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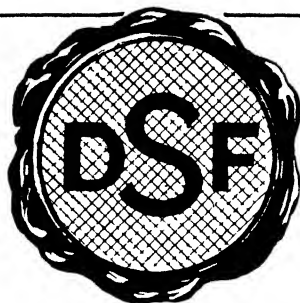


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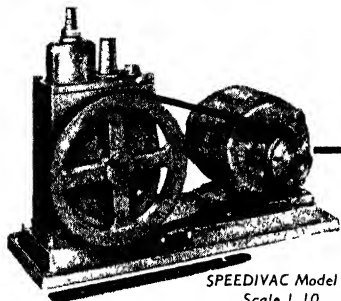
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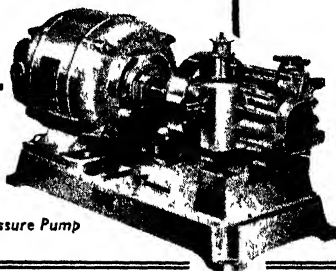
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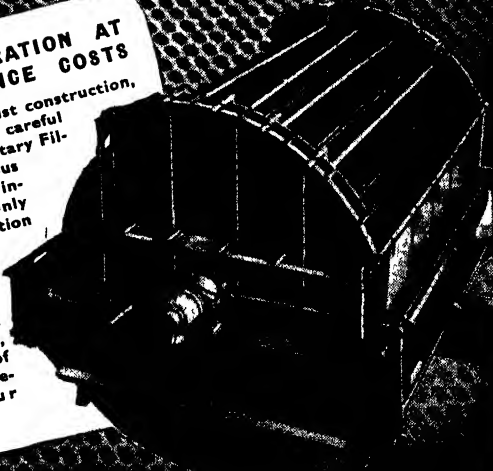
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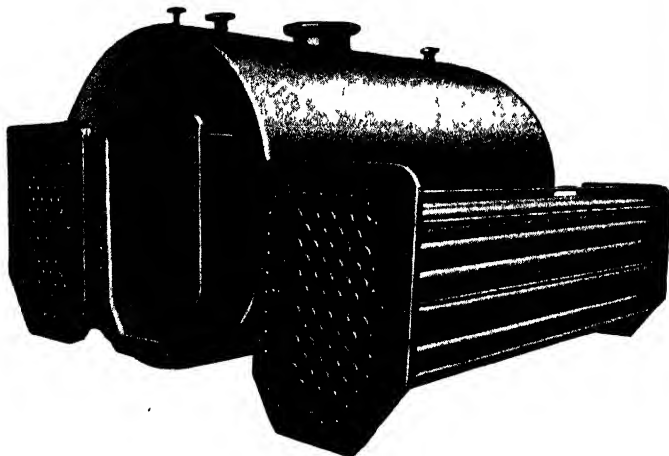
The widest possible view has been taken as to the meaning of the words "chemical composition"; besides including vitamins, amino-acids and other constituents of, less clear function (nucleotides, nitrogenous bases and so on), authors have given attention to the physical properties of the foods and also to the effect on them of cooking and processing. Based on a series of lectures given in war-time Britain, the seven parts of this book have much more than an ephemeral value.

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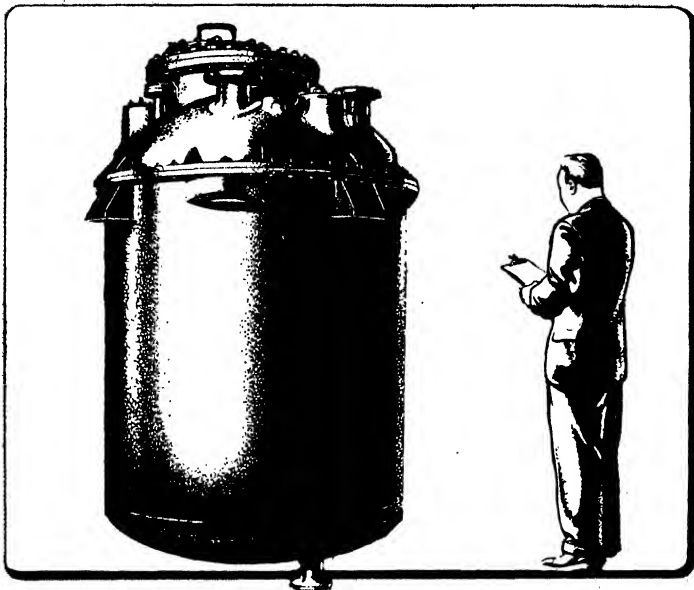
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action. Although all these forces operate in varying degrees to account for the departure from ideal behaviour of a gas such as hydrogen chloride, (a) and (e) alone are significantly involved for non-polar "permanent" gases, which accounts for the tendency to restrict the term van der Waals forces to term (a).

The calculation of these separate terms has frequently been made for comparison with the observed  $\Delta H$  values of hydrogen bonds. It is not difficult to obtain items (b) and (c) with sufficient accuracy, but the term (a) is far more difficult to evaluate except for simple molecules oriented at random, *i.e.*, in the gas phase; and much has still to be learnt about the correct representation of (e). For this reason the interpretation of the second virial coefficient  $B(T)$ , a function of temperature only, in the gas equation

$$PV = RT + P \cdot B(T) + P^2 \cdot C(T) + P^3 \cdot D(T) + \dots$$

is of especial significance. On the basis of this function, W. H. Stockmayer<sup>27</sup> has studied the molecular interactions in steam and ammonia. Allowing accurately for (b) and (c), and representing (e) as a power function of the intermolecular separation ( $r$ ), giving  $E_{\text{repl.}} = \lambda r^{-24}$ , he shows that the virial coefficients observed for steam from 400° to 750° K. require a molecular diameter  $\sigma = 2.76$  A.; and  $c_1 = 4.7 \times 10^{-59}$  erg-cm.<sup>6</sup> as the coefficient of  $r^{-6}$  in the energy expression for (a). These figures are in excellent agreement with other independent estimates. For ammonia, a less complete analysis assuming impenetrable molecules of diameter 3.18 A. (cf. N . . . N in solid ammonia, 3.38 A.<sup>28</sup>) gave  $c_1 = 7.8 \times 10^{-59}$  erg-cm.<sup>6</sup>, to be compared with the theoretical value  $c_1 = 7.0 \times 10^{-59}$  erg-cm.<sup>6</sup>.<sup>29</sup> Stockmayer's exponent in the repulsive energy term is far higher than that usually assumed. A later treatment<sup>30</sup> of the same data has taken the more commonly used inverse twelfth power for  $E_{\text{repl.}}$ ; the authors then extended the analysis in an interesting way. As the second virial coefficient arises only from factors governing collisions between two molecules, it can be related rigorously to the interaction energy of pairs of molecules in the collision state ( $\Delta E$ ). The values of  $\Delta E$  deduced for water and ammonia are 2510 and 1376 cal. per g.-mol. respectively. Such quantitative treatments as the foregoing are certain to contribute greatly to our knowledge of molecular interactions in general, and the results for water and ammonia are immediately relevant here, as hydrogen bonds play a large part in interpreting the behaviour, at least of the former.

C. F. Curtiss and J. O. Hirschfelder<sup>31</sup> have indicated a more indirect but more generally available method of determining second virial coefficients than that from an extended equation of state. This is done by

<sup>27</sup> *J. Chem. Physics*, 1941, **9**, 398.

<sup>28</sup> H. Mark and E. Pohland, *Z. Krist.*, 1925, **61**, 532.

<sup>29</sup> H. Margenau, *Rev. Mod. Physics*, 1939, **11**, 1.

<sup>30</sup> J. O. Hirschfelder, F. T. McClure, and I. F. Weeks, *J. Chem. Physics*, 1942, **10**, 201.

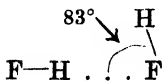
<sup>31</sup> *Ibid.*, p. 491.

rewriting the Clausius-Clapeyron equation for vapour pressure ( $P$ ) variation with temperature ( $T$ ) in the form

$$\frac{PV}{RT} = - \frac{\Delta H}{R \left(1 - \frac{V_L}{V}\right) d \ln P / d(1/T)}$$

where  $\Delta H$  is the latent heat per mole, and  $V_L$  and  $V$  are the molar volumes for liquid and vapour. The right-hand side is evaluated in terms of directly observed quantities to give  $PV/RT = 1 + \beta/V$ . The higher terms being neglected, the second virial coefficient is calculated from the value of  $\beta$ . Large negative values for  $\beta$  indicate increasing fractions of collision double molecules. This  $\beta$  term is, however, an *omnium gatherum* factor, including all the departures from ideality as well as errors of experiment and calculation. Taking it as a measure of the second virial coefficient, Curtiss and Hirschfelder calculate the fraction of double molecules in the vapours of a number of simple compounds. For methyl alcohol, ethyl alcohol, and benzene they are able to give values at three or more temperatures: from these it would appear that the interaction energies are  $5.1 \pm 0.3$ ,  $4.5 \pm 0.3$ , and  $2.2 \pm 0.8$  kcals. per g.-mol. of dimer respectively. The values are of the correct order of magnitude. It is clear that systematic determination of the Joule-Thomson coefficients for these and similar gases would give far greater precision to our knowledge of interaction energies for a phase where they could be theoretically analysed.

G. Briegleb<sup>32</sup> has made systematic calculations in electrostatic terms for hydrogen fluoride and its polymers. For this particular hydrogen halide he shows that the bonding energy in the monomer (365 kcals. per g.-mol.) is within 5% of that calculated for a simple polarised ionic binding.<sup>33</sup> This agrees with C. P. Smyth and N. B. Hannay's estimate<sup>34</sup> from the dipole moment that the "amount of ionic character" in hydrogen fluoride is intermediate between that in potassium iodide and potassium chloride. Using the electron-diffraction value<sup>35</sup> of the F . . . F distance in the polymers, a calculation of the terms (a), (b), (c), and (e)—the energy term of the last being taken as proportional to  $r^{-9}$ —on the basis of point dipoles gives  $\Delta E = 6.8$  kcals. for the dimer. For the short distances involved, such calculations are far more accurate when made by resolving the dipoles into their effective charges. With that procedure, Briegleb finds that the maximum interaction occurs when the components are almost perpendicular to one another (see inset). Emphasis is laid on the distortion of the H-F bond as compared with the monomer: a distortion for which the vibrational spectra provide substantial evidence. According as the H-F distance assumed in the dimer varies between 0.92 Å. (the value in the monomer) and 0.98 Å.,



<sup>32</sup> *Z. physikal. Chem.*, 1941, **B**, 51, 9.

<sup>33</sup> See also M. Born and W. Heisenberg, *Z. Physik*, 1924, **23**, 388; L. Pauling, *Proc. Roy. Soc.*, 1927, **A**, 114, 181.

<sup>34</sup> *J. Amer. Chem. Soc.*, 1946, **68**, 171.

<sup>35</sup> S. H. Bauer, J. Y. Beach, and J. H. Simons, *ibid.*, 1939, **61**, 19.

the calculated net electrostatic bonding energy varies from 9.5 to 5.5 kcal. per g.-mol. of monomer (cf. Table I). Similar calculations for the trimer  $(\text{HF})_3$  show a maximum interaction for the antiparallel arrangement of dipoles indicated by the electron-diffraction study as the probable structure of the chain polymer. The calculations suggest a decreasing average value of the bonding energy in going from dimer to trimer. Unfortunately, the vapour-density data are not sufficiently detailed to test this conclusion.<sup>7</sup>

Less complete electrostatic calculations have been attempted in a large number of other cases.<sup>36</sup> Verwey's calculation of the intermolecular forces in water may be singled out for further mention. A careful consideration of the charge distribution in the water molecule leads to a total attractive energy between 17.8 and 19.9 kcal. per g.-mol. for the known structure of ice. Use of the Born or the Born-Mayer form for the repulsive energy term gives a maximum range for the latter of 6.3–10.7 kcal. per g.-mol.: on the basis of data for the alkali halides the value 8.4 is chosen as the best estimate. The calculated latent heat of evaporation of ice is thus 9.4–11.5 kcal. per g.-mol. The experimental value is 10.8 in the same units. As the uncertainty in the London and in the repulsive energy term is of the order of at least 1 kcal., the agreement is as good as can be attained with our present knowledge.

In many other cases, apart from the uncertainty in estimating (a), the repulsive term (e) has been neglected, so that the result is simply the positive contribution of (b) plus (c) to the stabilisation energy. The quantitative significance of such results is thus in question. However, for the interaction energy in a simple case the terms (a), (b), (c), and (e) may be given a total representation  $E(r) = A/r^{12} - B/r^6 - C/r^3$ ; i.e., (e) is taken in the form  $A/r^{12}$ ; (b) =  $(-C/r^3)$ ; (a) + (c) =  $(-B/r^6)$ , in formal agreement with the leading members in the full expansion for these terms. If it then obtains that at equilibrium the contribution of the dipole-dipole term is twice that of the induction and dispersion forces combined, i.e.,  $C/r_{\text{eq.}}^3 = 2B/r_{\text{eq.}}^6$ —as is approximately found in a number of cases<sup>32, 36</sup>—then  $E(r_{\text{eq.}}) = -C/r_{\text{eq.}}^3$ , and the net interaction is equal to that contributed by the dipole term alone. It is to be hoped, however, that such approximations will not be necessary in future treatments. Where reasonably valid calculations can be made it appears that at least 70% of the hydrogen-bond energy can be ascribed directly to electrostatic effects. The Reporter is aware of only one explicit calculation of the resonance energy contribution to a hydrogen bond.<sup>37</sup>

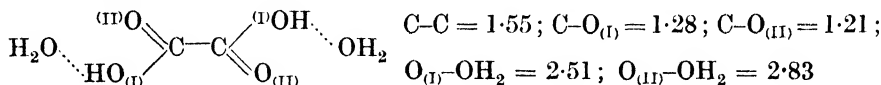
**X-Ray Studies.**—The results of crystal-structure determinations provided the first important structural evidence of the existence and extent of

<sup>36</sup> For instance: G. Briegleb, *Z. physikal. Chem.*, 1933, B, **23**, 105; K. Berger, *ibid.*, 1933, B, **22**, 283; 1935, B, **28**, 95; J. H. de Boer, *Trans. Faraday Soc.*, 1936, **32**, 10; M. Magat, *ibid.*, 1937, **33**, 714; *Ann. Physique*, 1936, **6**, 108; E. A. Moelwyn-Hughes, *J.*, 1938, 1243; H. Harms, *Z. physikal. Chem.*, 1939, B, **43**, 257; M. M. Davies, *Trans. Faraday Soc.*, 1940, **36**, 333; E. J. W. Verwey, *Rec. Trav. chim.*, 1941, **60**, 887.

<sup>37</sup> R. Gillette and A. Sherman, *J. Amer. Chem. Soc.*, 1936, **58**, 1135.

hydrogen bonding.<sup>38</sup> A large number of examples have since been studied in both the organic<sup>2</sup> and the inorganic field, and many summaries are available.<sup>39</sup> The results, in the form of abnormally low distances between atomic centres not otherwise bonded but between which a hydrogen bond may be located, are usually most precise for ionic structures. This generally results from the simpler units and greater crystalline symmetry of such compounds compared with purely organic structures. Among the more recent X-ray studies showing the importance of the hydrogen bond in inorganic compounds are those of solid hydrogen fluoride,<sup>40</sup> iodic acid,<sup>41</sup> and hydrazinium difluoride.<sup>42</sup>

Of the many organic structures revealing hydrogen bonding, that of oxalic acid dihydrate has been repeatedly studied<sup>43</sup> and for that reason alone deserves special mention. The most recent treatment<sup>44</sup> is also the most complete: the molecular unit can now be represented with the bond distances (in Å.):



The distance 2.51 Å., which is well established for this structure, appears to be the shortest hydrogen bridge known between oxygen atoms. Thus it is possible that an oxonium oxalate structure contributes significantly to the molecular state, but the normal value found for the C-C distance clearly precludes many of the "resonance structures" which have been discussed for this system.<sup>45</sup> It may be indicated that the C-O (1.28) and C=O (1.21) distances estimated by Davies and Sutherland for dimeric acetic acid are in agreement with the values found here, though this can best be taken as confirming the indirect spectroscopic estimates.

Robertson and Ubbelohde have studied with particular care the effect of deuterium substitution on the crystal structure of this and related hydrogen-bonded structures.<sup>46</sup> Whilst the electron distribution will remain unchanged by deuterium substitution, at least three possibly significant factors will vary: associated with the decreased zero-point energy there

<sup>38</sup> NaHF<sub>2</sub>: F. Rinne, H. Hentschel, and J. Leonhardt, *Z. physikal. Chem.*, 1922, **100**, 408; H<sub>2</sub>O: W. H. Bragg, *Proc. Physical Soc.*, 1922, **34**, 98.

<sup>39</sup> (a) L. Pauling, "The Nature of the Chemical Bond", Cornell Univ. Press, New York, 1939; (b) A. F. Wells, "Structural Inorganic Chemistry", Oxford Univ. Press, 1945.

<sup>40</sup> P. Gunther, K. Holm, and H. Strunz, *Z. physikal. Chem.*, 1939, *B*, **43**, 229.

<sup>41</sup> M. T. Rogers and L. Helmholz, *J. Amer. Chem. Soc.*, 1941, **63**, 278.

<sup>42</sup> M. L. Kronberg and D. Harker, *J. Chem. Physics*, 1942, **10**, 309.

<sup>43</sup> (a) W. H. Zachariasen, *Z. Krist.*, 1934, **89**, 442; (b) J. M. Robertson and I. Woodward, *J.*, 1936, 1817; (c) J. M. Robertson and A. R. Ubbelohde, *Proc. Roy. Soc.*, 1939, *A*, **170**, 222, 241; (d) R. Brill, C. Hermann, and Cl. Peters, *Naturwiss.*, 1939, 677; (e) J. M. Robertson, *Trans. Faraday Soc.*, 1940, **36**, 913.

<sup>44</sup> R. Brill, C. Hermann, and Cl. Peters, *Ann. Physik*, 1942, **42**, 257.

<sup>45</sup> See 43 (c) and 43 (e).

<sup>46</sup> See 43 (c) and A. R. Ubbelohde, *Trans. Faraday Soc.*, 1936, **32**, 525.

will be (i) a slight contraction of the O-H covalent bond distance, (ii) a diminished dipole moment, and, independently, (iii) a reduced ability of the deuterium, compared with hydrogen, to penetrate the potential hump separating two resonating structures. The first two changes will be very small, as shown for instance by the equilibrium distances in the isolated  $O \cdots O$  and  $O-D$  radicals being 0.9710 Å. and 0.969 Å. respectively.<sup>47</sup> The dipole moments should change in the same proportion. Thus the changes on deuterium substitution will provide an index of the contribution of protonic resonance to the hydrogen bond, for doubling the mass will greatly diminish the tunnel effect involved in this contribution. With their very precise comparisons of lattice parameters Robertson and Ubbelohde found no significant changes for phthalic acid and  $\alpha$ - and  $\beta$ -resorcinol on deuterium substitution. The lattice spacings were constant to about  $10^{-4}$  Å. For benzoic and succinic acids the isotope effect on the spacings was of a magnitude 0.0004 Å., about twice that amount in sodium hydrogen carbonate and greatest in oxalic acid dihydrate, where the spacing changes were some 0.004 Å. Taking the unit cell for  $(CO_2H)_2 \cdot 2H_2O$  as  $a = 6.120 \pm 0.020$ ,  $b = 3.600 \pm 0.010$ ,  $c = 12.030 \pm 0.030$ ,  $\beta = 106^\circ 12'$ , the results for  $(CO_2D)_2 \cdot 2D_2O$  gave  $a = 6.149$ ,  $b = 3.600$ ,  $c = 12.071$ ,  $\beta = 106^\circ 33'$ . These changes when interpreted as alterations of bond lengths are very small compared with that brought about by hydrogen bonding—i.e., a fall in  $O \cdots O$  distances from about 3.5 to 2.5 Å. It is a legitimate conclusion that deuterium substitution has proportionately very little effect on the bond conditions and that resonance in such structures is not energetically significant. Spectroscopic evidence strongly supports this conclusion.

A. R. Ubbelohde and I. Woodward have similarly studied the isotope effects in some acid phosphates<sup>48</sup> and the rôle of hydrogen bonds in Rochelle salt.<sup>49</sup> The crystal structure of the latter has been determined by C. A. Beevers and W. Hughes:<sup>50</sup> a hydrogen-bond length of  $2.56 \pm 0.05$  Å. was established. For this crystal, Ubbelohde and Woodward now suggest as an explanation of the lower Curie point (marking the onset of anomalous dielectric properties) that below that temperature the hydrogen bridge is symmetrical with the hydrogen at the mid-point, but at that temperature thermal expansion of the lattice reaches a critical value which causes the hydrogen to move into an unsymmetrical position. This would account for the sudden increase in dielectric susceptibility. The suggestion is an interesting one. If it were confirmed it would provide the first instance of a symmetrical bridge. However, it may be pointed out that Huggins's potential function,<sup>51</sup> used to support the suggestion, cannot be relied upon quantitatively as it would certainly predict a symmetrical bridge in  $KH_2PO_4$  (see below). Further, the change from a symmetrical to an unsymmetrical

<sup>47</sup> G. Herzberg, "Molecular Spectra and Molecular Structure," Vol. I, Prentice-Hall, Inc., New York, 1939.

<sup>48</sup> *Proc. Roy. Soc.*, 1942, A, 179, 399.

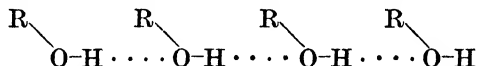
<sup>49</sup> *Ibid.*, 1946, A, 185, 448.

<sup>50</sup> *Ibid.*, 1941, A, 177, 251.

<sup>51</sup> M. L. Huggins, *J. Physical Chem.*, 1936, 40, 723.

bridge should be accompanied by an entropy change. This does not appear to be experimentally detectable at the Curie points in Rochelle salt,<sup>52</sup> and its absence provides a major obstacle to the acceptance of Ubbelohde and Woodward's picture.

The quantitative measure of X-ray diffraction is also able to give significant indications of molecular distribution in the liquid state. J. D. Bernal and R. H. Fowler's use of the data for water is well known.<sup>53</sup> Both methyl and ethyl alcohol have been carefully studied,<sup>54, 55</sup> Warren's treatment<sup>56</sup> of the intensities being used to give atomic distribution curves. At  $-75^\circ$  liquid methyl alcohol gave considerable detail, four diffraction rings being visible on the film. The pronounced peak in the radial distribution curve near 2.70 Å. corresponded to the  $O \cdots O$  distance for the hydrogen-bonded molecules. From the area of this peak it could be calculated that the number of near neighbours to each hydroxyl group averaged two, in agreement with the usual formulation of alcohol association :



For ethyl alcohol the maxima were not so clearly defined at  $-75^\circ$  but the O-H distance appeared to be 2.9 Å. in this case. When examined in the supercooled state<sup>57</sup> sharper maxima were again obtained which agreed well with Harvey's representation of the liquid.

*Entropy Studies.*—An interesting fact accounted for by the dissymmetry in a particular hydrogen bridge is the "residual entropy" of ice. L. Pauling gave the interpretation of this phenomenon.<sup>58</sup> In the solid the oxygen atoms are in tetrahedral co-ordination and each hydrogen is about 0.95 Å. from one oxygen and 1.81 Å. from another. Above  $200^\circ \text{K}$ . the dielectric constant of ice is of the same magnitude as that of water, showing that its dipoles (O-H bonds) can orient themselves with considerable freedom. At lower temperatures the solid "freezes" into one of the large number ( $W$ ) of configurations possible for the location of the hydrogen atoms. The existence of these equivalent locations introduces a randomness not otherwise anticipated and so an entropy term  $\Delta S = k \ln W$ . As the energy differences between the various configurations are very small, no transition to a particular group of them occurs in the range of temperatures for which specific heats have been measured. Accordingly, the solid retains this item of entropy in the lowest observed range.

A simplified calculation of  $\Delta S$  can be given in terms of the six orient-

<sup>52</sup> C. C. Stephenson and J. G. Hooley, *J. Amer. Chem. Soc.*, 1944, **66**, 1397.

<sup>53</sup> *J. Chem. Physics*, 1933, **1**, 515.

<sup>54</sup> W. H. Zachariasen, *ibid.*, 1935, **3**, 158; G. G. Harvey, *ibid.*, 1938, **6**, 111; 1939, **7**, 878.

<sup>55</sup> W. S. Pierce and D. P. MacMillan, *J. Amer. Chem. Soc.*, 1938, **60**, 779.

<sup>56</sup> *J. Appl. Physics*, 1937, **8**, 645.

<sup>57</sup> A. Prietzschk, *Z. Physik*, 1941, **117**, 482.

<sup>58</sup> *J. Amer. Chem. Soc.*, 1935, **57**, 2680.

ations possible for a single  $\text{H}_2\text{O}$  molecule (Fig. 1). The chance that the adjacent molecules ( $a$ ,  $b$ ,  $c$ ,  $d$ ) will permit any particular one of these ( $Oa + Ob$ , say) is  $1/4$ , inasmuch as the chance of  $a$  having one of its hydrogens along  $Oa$  is  $1/2$ , and similarly for  $b$  along  $Ob$ . Thus the total number of configurations for an assembly of  $N$  molecules is  $W = (6/4)^N$ : whence  $\Delta S = R \cdot \ln (3/2) = 0.805$  cal. per g.-mol. per  $^\circ\text{K}$ .<sup>59</sup> For the vapour under standard conditions the spectroscopic value of the entropy is 45.101 units: the thermal value calculated from specific and latent heats, etc., is 44.23 units: the difference, 0.87 unit, is a measure (including experimental errors

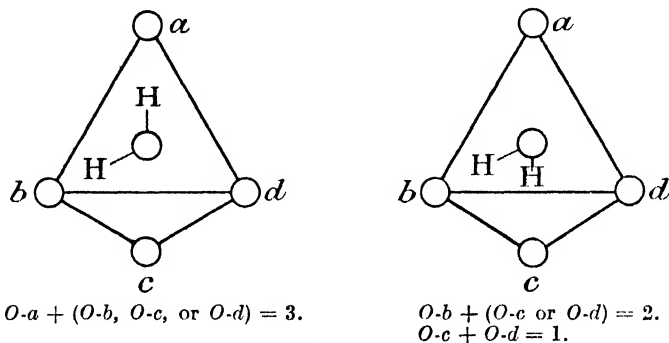


FIG. 1.

in the separate figures quoted) of the excess entropy retained by the solid at the lowest temperatures. The agreement with the calculated value is very satisfactory.

It is clear that this entropy term depends upon the unsymmetrical location of the hydrogen in the  $\text{O} \cdots \text{O}$  bridge. The occurrence of this term and its precise determination in a number of instances provides an interesting refutation of the supposition<sup>60</sup> frequently repeated<sup>39b</sup> that in a number of short hydrogen bonds, as normally observed, the hydrogen is symmetrically located. Among the structures where an excess entropy in the solid has been satisfactorily explained on lines similar to those for ice are a series of inorganic salts studied by C. C. Stephenson and his collaborators. These are  $\text{KH}_2\text{PO}_4$ ,<sup>61</sup>  $\text{KH}_2\text{AsO}_4$ ,<sup>62</sup>  $(\text{NH}_4)_2\text{H}_2\text{PO}_4$ ,<sup>63</sup>  $(\text{NH}_4)_2\text{H}_2\text{AsO}_4$ ,<sup>64</sup>  $\text{Ag}_3\text{H}_3\text{IO}_6$ ,<sup>65</sup>  $(\text{NH}_4)_2\text{H}_3\text{IO}_6$ ;<sup>66</sup> K. S. Pitzer and L. V. Coulter studied  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ .<sup>67</sup>

There is one important difference between these cases and ice. If we take  $\text{KH}_2\text{PO}_4$  as an example ( $\text{O} \cdots \text{O}$  bridge =  $2.54 \pm 0.05 \text{ \AA}$ ),<sup>68</sup> the possible

<sup>59</sup> For a more complete treatment, see J. C. Slater, *J. Chem. Physics*, 1941, **9**, 16.

<sup>60</sup> J. D. Bernal and H. D. Megaw, *Proc. Roy. Soc.*, 1935, **A**, **151**, 384.

<sup>61</sup> See (52) and other references given there.

<sup>62</sup> C. C. Stephenson and A. C. Zettlemeyer, *J. Amer. Chem. Soc.*, 1944, **66**, 1402.

<sup>63</sup> *Idem*, *ibid.*, p. 1405.

<sup>64</sup> C. C. Stephenson and H. E. Adams, *ibid.*, p. 1409.

<sup>65</sup> *Idem*, *ibid.*, p. 1412.

<sup>66</sup> C. C. Stephenson, *J. Chem. Physics*, 1941, **9**, 379.

<sup>67</sup> *J. Amer. Chem. Soc.*, 1938, **60**, 1310.

<sup>68</sup> S. B. Hendricks, *Amer. J. Sci.*, 1927, **14**, 269.

number of configurations is the same as in ice, two hydrogens being associated with each phosphate tetrahedron. However, the configurations are not now all of the same energy and there is a transition temperature ( $121.97^\circ \pm 0.05^\circ \text{K.}$ ) below which a unique distribution is assumed by the hydrogens. In this particular configuration all the  $(\text{H}_2\text{PO}_4)^-$  dipoles are oriented parallel to the  $c$ -axis, giving the crystal its spontaneous polarisation along this axis below the transition temperature. Accordingly, the additional entropy appears only above the transition point and so its value can be directly measured. For  $\text{KH}_2\text{PO}_4$  the results gave  $\Delta S = 0.74 \pm 0.06 \text{ cal. deg.}^{-1} \text{ mole}^{-1}$ . Slater's treatment<sup>69</sup> predicts a first-order change  $\Delta S = \frac{1}{2}R \ln 2 = 0.69 \text{ cal. deg.}^{-1} \text{ mole}^{-1}$  at the transition temperature, followed by a further gradual increase to the total  $\Delta S = R \ln (3/2) = 0.805 \text{ cal. deg.}^{-1} \text{ mole}^{-1}$  at higher temperatures. For the distribution of the three hydrogens attached to the  $\text{IO}_6$  octahedron in  $\text{Ag}_2\text{H}_3\text{IO}_6$ , the value predicted for the transition temperature is  $\Delta S = \frac{3}{4}R \ln 3 = 1.64$  entropy units, followed by a further increment making the total  $\Delta S = R \ln (5/2) = 1.82$  entropy units. Direct measurements gave  $\Delta S = 1.60 \pm 0.10$  entropy units at the transition point.

J. O. Halford<sup>69</sup> has used the entropies of the dimeric forms of formic and acetic acid to estimate a force constant for the hydrogen bond in these molecules. The value so found,  $k = 4.0 \pm 1.0 \times 10^4$  dynes per cm., will be further mentioned in the section on infra-red spectra.

*Infra-red Studies.*—Absorption studies in the second overtone region for the O-H group ( $\lambda \sim 9700 \text{ \AA.}$ ) allowed R. Freymann in 1932 to demonstrate clear spectroscopic changes on molecular association.<sup>70</sup> Similar effects in the fundamental region ( $\lambda \sim 2.75\mu$ ;  $\nu \sim 3640 \text{ cm.}^{-1}$ ) were found by J. Errera and P. Mollet in 1936.<sup>71</sup>

The form taken by the greater part of subsequent infra-red work on this topic has been to study the change in the X-H valency vibration when it is involved in a hydrogen bond, X-H . . . Y. Other spectroscopic changes will be mentioned later. By virtue of the single covalency and small mass of the hydrogen, the X-H vibrations can justifiably be treated as involving only the force constants for that bond, an approximation which is further improved in those cases (*e.g.*, O-H) where the valency angle of X is roughly  $90^\circ$ . The stronger the dipolar character of X-H the more intense these absorptions will be, so that it is comparatively easy to follow them over wide changes of state. As a change in frequency for the peak absorption of a few wave numbers ( $\text{cm.}^{-1}$ ) is readily measured, it will be understood that the method is a sensitive one for following slight changes in the X-H bond.

For the X-H stretching vibration the results in such cases as the progressive association of the alcohols and phenol<sup>72</sup> are comparatively simple.

<sup>69</sup> *J. Chem. Physics*, 1946, **14**, 395.

<sup>70</sup> *Compt. rend.*, 1932, **195**, 39.

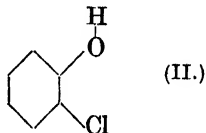
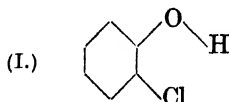
<sup>71</sup> *Nature*, 1936, **138**, 882; see also C. E. H. Bawn, *J.*, 1932, 1189.

<sup>72</sup> *Inter alia*: (a) J. J. Fox and A. E. Martin, *Proc. Roy. Soc.*, 1937, **A**, **162**, 419; (b) *idem*, *Trans. Faraday Soc.*, 1940, **36**, 897; (c) R. Freymann, *Compt. rend.*, 1937, **204**, 1063; (d) E. L. Kinsey and J. W. Ellis, *J. Chem. Physics*, 1937, **5**, 399; (e) R. M. Badger and E. H. Bauer, *ibid.*, p. 938; (f) J. Errera, R. Gaspart, and H. Sack, *ibid.*, 1940, **8**, 63.



The sharp absorption due to monomeric ethyl alcohol molecules at  $3638\text{ cm}^{-1}$  ( $2.75\text{ }\mu$ ) is accompanied by a much broader absorption centred at about  $3325\text{ cm}^{-1}$  when association occurs. The decreased frequency ( $\Delta\nu = 310\text{ cm}^{-1}$ ) clearly means that the hydroxyl group in the associated molecule participates in an interaction which reduces the restoring force in the O-H bond. A similar change occurs on the association of carboxyl groups<sup>73</sup> when the sharp absorption at  $3530\text{ cm}^{-1}$  is replaced by an exceedingly broad band extending from about  $3330$  to  $2380\text{ cm}^{-1}$ . Davies and Sutherland<sup>73</sup> have emphasised the occurrence of changes, albeit smaller, in the C=O and C-O bonds at the same time. The formation of hydrogen bonds having nitrogen at X or Y has also been studied by these methods.<sup>74</sup>

Spectroscopic measurements have provided the means of establishing many interesting cases of intramolecular interaction of the same general nature as hydrogen-bond formation. This aspect has been the particular concern of Wulf and his co-workers.<sup>75</sup> One of the simplest examples is that of *o*-chlorophenol, where the explanation of two sharp absorptions observed for the monomeric molecules<sup>75a</sup> was given by Pauling<sup>76</sup> in terms of the *cis*-(I) and *trans*-(II) configurations stabilised by the energy minima



for the hydrogen at the two positions in the benzene-ring plane. The study of the temperature variation of these absorptions in solution<sup>77</sup> and in the vapour<sup>78</sup> has confirmed this interpretation, although the occurrence of resonance with a quinonoid form of the phenolic group (indicated by Pauling) does not appear to be a necessary part of the phenomenon.<sup>79</sup> Davies has found that the spectroscopic evidence points to the same interaction being the stabilising factor in chloral and bromal hydrates<sup>80</sup> and in other compounds having a stable  $>\text{C}(\text{OH})_2$  group.<sup>79</sup>

<sup>73</sup> (a) R. M. Badger and S. H. Bauer, *J. Chem. Physics*, 1937, **5**, 605; (b) M. M. Davies and G. B. M. Sutherland, *ibid.*, 1938, **6**, 755, 767; (c) refs. (11), (13); (d) R. Hofstadter, *J. Chem. Physics*, 1938, **6**, 540; 1940, **8**, 252; (e) A. M. Buswell, W. H. Rodebush and M. F. Roy, *J. Amer. Chem. Soc.*, 1938, **60**, 2239.

<sup>74</sup> J. W. Ellis, *J. Chem. Physics*, 1939, **7**, 862; *Physical Rev.*, 1939, **55**, 1098; A. M. Buswell, J. R. Downing, and W. H. Rodebush, *J. Amer. Chem. Soc.*, 1940, **62**, 2759; H. W. Thompson and G. P. Harris, *J.*, 1944, 301; W. Gordy, *J. Chem. Physics*, 1939, **7**, 167; W. Gordy and S. C. Stanford, *J. Amer. Chem. Soc.*, 1940, **62**, 497.

<sup>75</sup> (a) O. R. Wulf and U. Liddel, *ibid.*, 1935, **57**, 1464; (b) O. R. Wulf, U. Liddel, and S. B. Hendricks, *ibid.*, 1936, **58**, 2287; (c) O. R. Wulf and L. S. Deming, *J. Chem. Physics*, 1938, **6**, 702; (d) O. R. Wulf and E. J. Jones, *ibid.*, 1940, **8**, 745; (e) O. R. Wulf, E. J. Jones, and L. S. Deming, *ibid.*, p. 753.

<sup>76</sup> L. Pauling, *J. Amer. Chem. Soc.*, 1936, **58**, 94.

<sup>77</sup> M. M. Davies, *Trans. Faraday Soc.*, 1938, **34**, 1427.

<sup>78</sup> L. R. Zumwalt and R. M. Badger, *J. Chem. Physics*, 1939, **7**, 87; *J. Amer. Chem. Soc.*, 1940, **62**, 305.

<sup>79</sup> M. M. Davies, *Trans. Faraday Soc.*, 1940, **36**, 1114.

Leaving these qualitative effects, we turn to the many studies of association equilibria by means of absorption intensities.<sup>81</sup> It has been possible to deduce equilibrium constants and, from measurements at different temperatures, heats of association have been calculated. Very careful intensity measurements are required if the latter values are to be accurate<sup>82</sup> and, particularly for solutions, a check should be made of any temperature change of absorption coefficient. It is noteworthy that Hoffmann<sup>82</sup> found that, whilst the extinction coefficient for the wavelength of maximum absorption varies from one alcohol to another, the integral absorption for the monomeric O-H band bears a constant relation to the number of free alcohol molecules. The association of alcohols and phenols<sup>72a, b, and f</sup> proceeds *via* the dimer to aggregates of increasing complexity, only the dimer being individually distinguishable among the polymers. A significant point in these treatments is that the monomeric molecules alone are found to contribute to the "free" O-H absorption.<sup>83</sup> This

suggests that the usual representation of the alcohol dimer is unsatisfactory insofar as it shows one of the hydroxyl groups apparently "free". Fox and Martin,<sup>72b</sup> however, believe that they have found the corresponding absorption for the benzyl alcohol dimer at  $2.77\mu$ , compared with  $2.765\mu$  for the free hydroxyl of the monomer. It should be mentioned that the quantitative analysis of the absorptions shows, in some cases at least, that only small fractions of dimer molecules are present at any stage; thus, for *tert.*-butyl alcohol, the results are in accurate agreement with a simple monomer  $\rightleftharpoons$  trimer equilibrium;<sup>82</sup> whilst, despite pronounced association, little of the dimer can be detected in solutions of ethyl, propyl, or butyl alcohol in carbon tetrachloride.<sup>17a, 84</sup>

It will be clear that the first datum provided by the infra-red studies is the change in frequency ( $\nu$ ) of the X-H vibration. This can be accurately related to the change in the force constant ( $k$ ) for the covalent bond. To deduce the accompanying change in structure, *i.e.*, the change in X-H bond length, one of the empirical equations connecting force constant and internuclear distance ( $r$ ) must be used.<sup>85</sup> The physical basis for these relations has been elucidated by G. B. B. M. Sutherland.<sup>86</sup> Treating X-H as a "diatomic" system, Badger's equation yields

$$\frac{dr}{r} = -\frac{1}{3} \frac{dk}{k} = -\frac{2}{3} \frac{d\nu}{\nu}$$

<sup>80</sup> M. M. Davies, *Trans. Faraday Soc.*, 1940, **36**, 333.

<sup>81</sup> See, *e.g.*, 72 (a), (b), (e), (f); 73 (b), (c).

<sup>82</sup> E. G. Hoffmann, *Z. physikal. Chem.*, 1943, **B**, **53**, 185.

<sup>83</sup> See, especially, H. Kempton and R. Mecke, *ibid.*, 1940, **B**, **46**, 229; E. G. Hoffmann, (82).

<sup>84</sup> J. Kreutzer and R. Mecke, *ibid.*, 1941, **B**, **49**, 309.

<sup>85</sup> R. M. Badger, *J. Chem. Physics*, 1934, **2**, 128; 1935, **3**, 710; C. H. D. Clark, *Trans. Faraday Soc.*, 1941, **37**, 299, and earlier papers.

<sup>86</sup> *Proc. Indian Acad. Sci.*, 1938, **8**, 341; see also, C. K. Wu and C. T. Yang, *J. Physical Chem.*, 1944, **48**, 295.

This gives a maximum change of the O-H bond length from 0.98 to 1.12 Å. on association of two carboxyl groups, the actual change probably being a good deal less. However, even this value leaves the hydrogen bridge in the dimer unsymmetrical, a conclusion fully confirmed by the other absorptions of the structure.<sup>73b</sup> The interpretation of the electron-diffraction results has been brought into agreement with this picture.<sup>87</sup> If a molecularly symmetrical structure X-H-X were to be produced by a potential minimum for the hydrogen half-way between the bonded atoms, the X-H valency vibration would disappear from the infra-red spectrum; this has not, as yet, been found to occur. In a number of intramolecular instances the X-H frequency is shifted to a region where it overlaps and is partly masked by other absorptions of the molecule: examples are *o*-nitrophenol, salicylaldehyde, and salicylate esters.<sup>88</sup> The chances of overlapping are increased in the overtone regions and this partly accounts for the failure to detect the shifted absorptions in some early work by Wulf and his associates.<sup>89</sup> A comparison of absorption intensities with similar compounds in which no hydrogen bond occurs will help to locate the shifted frequency: or, better, deuterium substitution will often place the X-D frequencies in a region where they are free from overlapping bands.

TABLE II.

Molecule.	HCl.	CH <sub>4</sub> .	H <sub>2</sub> S.	C <sub>6</sub> H <sub>6</sub> .	NH <sub>3</sub> .
Gas (cm. <sup>-1</sup> ) .....	2889	2914	2611	3099	3334
Liquid (cm. <sup>-1</sup> ) .....	2785	2909	2573	3090	3300
Percentage change from gas .....	-3.6	-0.17	-1.5	-0.3	-1.0
Solid (cm. <sup>-1</sup> ) .....	2744	2906	2553	3089	3303
	2701				
Percentage change from gas .....	-5.0	-0.27	-2.2	-0.3	-0.9
	-6.5				

Molecule.	HCN.		SbCl <sub>3</sub> .	BCl <sub>3</sub> .			PCl <sub>3</sub> .
(Gas (cm. <sup>-1</sup> ) .....	2089	3312	3823	924	958	996	355
Liquid (cm. <sup>-1</sup> ) .....	2094	3213	360	902	946	989	314
Percentage change from gas .....	0.2	-3.0	-5.8	-2.4	-1.2	-0.7	-11.6

In any discussion of these frequency shifts it must be emphasised that similar, although generally smaller, changes are found in a wide variety of circumstances not connected with hydrogen-bond formation. The most common of these is change of state, and Table II shows some typical examples: not all the frequencies of a given molecule are quoted, and some of the data are derived from Raman spectra.<sup>90</sup>

Such changes have usually been ascribed to electrostatic forces and the

<sup>87</sup> Cf. L. Pauling and L. O. Brockway, *Proc. Nat. Acad. Sci.*, 1934, **20**, 336; J. Karle and L. O. Brockway, *J. Amer. Chem. Soc.*, 1944, **66**, 571.

<sup>88</sup> A. M. Buswell, V. Deitz, and W. H. Rodebush, *J. Chem. Physics*, 1937, **5**, 501, 726.

<sup>89</sup> *J. Amer. Chem. Soc.*, 1933, **55**, 3574; and 75(a).

<sup>90</sup> See, also, G. Herzberg, "Infrared and Raman Spectra of Polyatomic Molecules," D. Van Nostrand Co., New York, 1945.

distortion of dipoles in the condensed phase.<sup>91</sup> What is important for the present purpose is that changes in absorption frequencies cannot be indiscriminately ascribed to specific "hydrogen bonding."<sup>92</sup> This is particularly to be noted for changes on going from one solvent to another: such facts as the appearance of the monomeric O-H absorption of benzyl alcohol at 3618, 3610, and 3571  $\text{cm}^{-1}$  in carbon tetrachloride, chloroform, and benzene solutions respectively<sup>93</sup> suggest that quite general factors may play an important part. Further, it is not permissible to deduce an increase or decrease in bonding energy as an immediate consequence of an increase or decrease in frequency. The latter change only relates directly to the force constant, *i.e.*, to the slope of the potential curve near the minimum, and it does not determine a change in the depth of the potential curve. That changes occur in the form of the latter on hydrogen bonding is probable from the different anharmonicities calculated for the free and hydrogen-bonded X-H vibrations.<sup>80</sup> Unfortunately, only for such cases as *o*-chlorophenol can this point be readily tested and even then data of mixed origin have to be combined. Owing to the great uncertainty in locating the centre of the association band in the different overtones, it is impossible to come to any conclusion in the case of the carboxylic acids, but data (again mixed) for hydrogen fluoride<sup>94</sup> suggest differences in anharmonicity between monomer and polymer.<sup>95</sup>

Accordingly, it is not surprising that, although several attempts have been made, no satisfactory relation has been found between the frequency changes on hydrogen bonding and the  $\Delta H$  values for the bond. Indeed, there are no very plausible grounds for anticipating a simple relation between the latter factor—which is the one usually measured by chemists and the one of most significance in their operations—and the changes of the potential field in the molecule. At best a correlation between  $\Delta H$  and  $\Delta \nu$  for similar groups involved in hydrogen bonds might be expected: as a particular instance, if the same  $\Delta \nu/\nu$  values are found for an OH and OD compound, it is reasonable to assume that the  $\Delta H$  values will be equal. R. M. Badger and S. H. Bauer, despite the limitations of which they were well aware, have indicated a rough empirical relation of  $\Delta H$  with  $\Delta \nu$  for bonds involving the hydroxyl group.<sup>96</sup> The form of the relation has been modified by Badger.<sup>97</sup>

A significant use of the frequency changes can be made by comparing them for hydrogen and deuterium bonds. For  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  in the vapour

<sup>91</sup> (a) W. West and R. T. Edwards, *J. Chem. Physics*, 1937, **5**, 14; (b) W. West, *ibid.*, 1939, **7**, 795; (c) J. R. Nielsen and N. E. Ward, *ibid.*, 1942, **10**, 81; (d) H. Braune and G. Engelbrecht, *Z. physikal. Chem.*, 1932, **B**, **9**, 303.

<sup>92</sup> See, also, A. M. Buswell, J. R. Downing, and W. H. Rodebush, *J. Amer. Chem. Soc.*, 1939, **61**, 3252.

<sup>93</sup> M. M. Davies, *J. Chem. Physics*, 1940, **8**, 577.

<sup>94</sup> (a) A. M. Buswell, R. L. Maycock, and W. H. Rodebush, *ibid.*, p. 362; (b) A. L. Wahrhaftig, *ibid.*, p. 349.

<sup>95</sup> See, however, R. M. Badger, *ibid.*, 1940, **8**, 288.

<sup>96</sup> *Ibid.*, 1937, **5**, 839.

<sup>97</sup> *Ibid.*, 1940, **8**, 288.

the unsymmetrical valence frequencies are 3756 and 2789  $\text{cm}^{-1}$ , respectively; in the liquids these become 3400 and 2507  $\text{cm}^{-1}$ .<sup>98</sup> The values of  $100 \Delta\nu/\nu$  for the hydrogen bonding accompanying the change of phase are thus 9.5 and 9.9 respectively. Some further examples are given in Table III.

The changes in hydrogen chloride may not, of course, be associated with a typical hydrogen bond. Whilst the figures quoted are not all of high accuracy (partly owing to the difficulty in locating the centre of the association bands) they suffice to show that there is no substantial difference resulting from deuterium substitution. It has already been emphasised that any contribution due to a resonance term in the bond energy would be greatly changed by doubling the mass of the hydrogen: the conclusion is, therefore, that in these cases this contribution is of very small proportions.

A fairly complete analysis of a hydrogen bond structure is provided by J. A. A. Ketelaar's study of the  $\text{KHF}_2$ ,  $\text{KDF}_2$  absorptions and reflections in the infra-red range 1—16  $\mu$ .<sup>3</sup> His interpretation of the observed frequencies is that they are nearly all combinations of the "hydrogen" or unsymmetric valency vibration ( $\nu_3$ ) with the infra-red inactive symmetrical vibration ( $\nu_1$ ) (see Fig. 2). The failure to detect  $\nu_1$  in the Raman spectrum

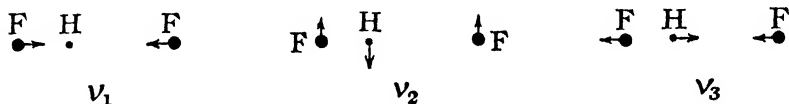


FIG. 2.

of these salts<sup>5</sup> is related to the small polarisability of the fluoride ion and the preponderatingly ionic character of the bond. The most important feature of the spectrum is the splitting of the fundamental  $\nu_3$  absorption into two components at 1222  $\text{cm}^{-1}$  and 1450  $\text{cm}^{-1}$  in  $(\text{HF}_2)^-$  and at 891  $\text{cm}^{-1}$  and 1046  $\text{cm}^{-1}$  in  $(\text{DF}_2)^-$ . Ketelaar assigns this doubling, which is consistently observed in the overtones and combinations of  $\nu_3$ , to a double minimum in the curve showing the potential energy of the hydrogen atom as a function of the distance from the fluorines. Using the treatment first evolved by G. E. Uhlenbeck and D. M. Dennison<sup>6</sup> for the  $\text{NH}_3$  molecule, he has drawn this potential curve for  $(\text{HF}_2)^-$ . The minima are estimated to be separated by 0.7 Å. and a potential hump of 2570  $\text{cm}^{-1}$ , equivalent to 7.3 kcal. per g.-mol. The F-F distance being taken as 2.26 Å.,<sup>7</sup> the two

<sup>98</sup> (a) E. F. Barker and W. W. Sleator, *J. Chem. Physics*, 1935, **3**, 660; (b) W. Gordy, *ibid.*, 1939, **7**, 93; (c) J. J. Fox and A. E. Martin, *Proc. Roy. Soc.*, 1940, **A**, **174**, 234.

<sup>99</sup> See (13).

<sup>1</sup> Ref. (90), p. 335.

<sup>3</sup> G. Bosschieter, *J. Chem. Physics*, 1937, **5**, 992.

<sup>2</sup> *Rec. Trav. chim.*, 1941, **60**, 523.

<sup>4</sup> E. Lee, G. B. B. M. Sutherland, and C. K. Wu, *Proc. Roy. Soc.*, 1940, **A**, **176**, 493.

<sup>5</sup> J. A. A. Ketelaar, ref. (3); L. A. Woodward and H. J. V. Tyrrell, *Trans. Faraday Soc.*, 1942, **38**, 513.

<sup>6</sup> *Physical Rev.*, 1932, **39**, 938.

<sup>7</sup> X-Ray result: L. Helmholz and M. T. Rogers, *J. Amer. Chem. Soc.*, 1939, **61**, 2590,

TABLE III.

$\text{C}_2\text{H}_5\text{CO}_2\text{H} \}$ $\text{CD}_3\text{CO}_2\text{H}$	$\text{C}_2\text{H}_5\text{CO}_2\text{D} \}$ $\text{CD}_3\text{CO}_2\text{D}$	$\text{CH}_3\text{CO}_2\text{D} \}$ $\text{CD}_3\text{CO}_2\text{D}$	$\text{CH}_3\text{CO}_2\text{D} \}$ $\text{CD}_3\text{CO}_2\text{D}$
Monomer	Monomer	Monomer	Monomer
Dimer	Dimer	Dimer	Dimer
$100\Delta\nu/\nu$	$100\Delta\nu/\nu$	$100\Delta\nu/\nu$	$100\Delta\nu/\nu$
$\text{CH}_3\text{OH}^1$	$\text{CH}_3\text{OD}^1$	$\text{CH}_3\text{OD}^1$	$\text{CH}_3\text{OD}^2$
Monomer	Monomer	Monomer	Monomer
Associated	Associated	Associated	Associated
$100\Delta\nu/\nu$	$100\Delta\nu/\nu$	$100\Delta\nu/\nu$	$100\Delta\nu/\nu$
$\text{HF}^{2,4}$	$\text{DF}$	$\text{DF}$	$\text{DF}$
$(\text{HF}_2)^{-3}$	$(\text{DF}_2)^{-3}$	$(\text{DF}_2)^{-3}$	$(\text{DF}_2)^{-3}$
$100\Delta\nu/\nu$	$100\Delta\nu/\nu$	$100\Delta\nu/\nu$	$100\Delta\nu/\nu$
$\text{HCl}^4$	$\text{DCI}$	$\text{DCI}$	$\text{DCI}$
Gas	Gas	Gas	Gas
Solid	Solid	Solid	Solid
$100\Delta\nu/\nu$	$100\Delta\nu/\nu$	$100\Delta\nu/\nu$	$100\Delta\nu/\nu$

2675 cm.<sup>-1</sup>  
2300 cm.<sup>-1</sup>  
14.02720 cm.<sup>-1</sup>  
2494 cm.<sup>-1</sup>  
8.32870 cm.<sup>-1</sup>  
968 cm.<sup>-1</sup>  
66.32091 cm.<sup>-1</sup>  
1965 cm.<sup>-1</sup>  
6.02721 cm.<sup>-1</sup>  
2515 cm.<sup>-1</sup>  
7.6Monomer  
Associated

minima are then at about 0.78 Å. and 1.48 Å. from any one fluorine. (In HF gas the internuclear separation is 0.917 Å.: the reality of the decrease to 0.78 Å. can only be established after a consideration of a number of possible errors.) To interpret the observed splitting in the absorptions involving  $\nu_3$ , Ketelaar takes a separation between the symmetrical and unsymmetrical ground states of 25  $\text{cm}^{-1}$ , and a splitting of 185  $\text{cm}^{-1}$  for the first vibrational level. Accordingly, the energy difference between the two ground states is  $h\Delta\nu$ , where  $\Delta\nu \sim 25 \text{ cm}^{-1}$ , *i.e.*, about 0.07 kcal. per g.-mol. As one level will be as much above as the other is below the energy level that would prevail in the absence of resonance splitting, it appears that the resonance contribution to the stabilisation of  $(\text{HF}_2)^-$  is of the order 0.04 kcal. per g.-mol. There is every indication that the hydrogen bond in  $(\text{HF}_2)^-$  is one of the strongest known: compare 100  $\Delta\nu/\nu$  on the formation of  $(\text{HF})_n$  and  $(\text{HF}_2)^-$ , *i.e.*, 13.4 and 66.3. In the absence of a direct determination, Ketelaar estimates the bonding energy  $\text{F}^- + \text{HF} \longrightarrow (\text{HF}_2)^- + Q$  to have a value for  $Q$  between 30 and 50 kcal. per g.-ion. Despite its small energetic significance, it is important that protonic resonance has been established in the structure, for previously only in  $\text{NH}_3$  and  $\text{PH}_3$  (where it corresponds to an inversion of the pyramid) have spectroscopic methods clearly shown it to occur. Although decreased as compared with  $(\text{HF}_2)^-$ , the resonance splitting is surprisingly large in  $(\text{DF}_2)^-$ , *e.g.*, for the vibrational transition ( $\nu_3$ )  $0 \longrightarrow 1$ , the separations are 228  $\text{cm}^{-1}$  and 155  $\text{cm}^{-1}$  respectively. This large value is presumably due to the first vibrational level lying near the top of the barrier. G. Glockler and G. E. Evans<sup>8</sup> have given an analysis of the same structure based on the assumptions that  $\nu_3 = 3720 \text{ cm}^{-1}$  and that the resonance splitting was 111  $\text{cm}^{-1}$  (*cf.* 228  $\text{cm}^{-1}$  above). These figures were derived from a far less complete study<sup>9a</sup> of the absorptions than Ketelaar's and, despite some unexpected features in his assignments, Ketelaar's treatment is the more satisfactory.

Buswell, Maycock, and Rodebush<sup>9a</sup> have measured the absorptions of gaseous hydrogen fluoride. The dilute gas has the normal vibrational band with rotational lines centred at 3960  $\text{cm}^{-1}$ . As the pressure increases up to atmospheric, a broad band extending from 3800 to 3150  $\text{cm}^{-1}$  appears and is clearly due to the associated molecules  $(\text{HF})_n$ . Although only dilute solutions (0.01M) in carbon tetrachloride could be examined, the authors draw attention to the particular weakness of the association features observed there. In view of the exceptional polarity of the HF molecule this may result from a strong dipole-induced dipole interaction with (*i.e.*, solvation by) the carbon tetrachloride.

As G. B. B. M. Sutherland has clearly indicated,<sup>9</sup> the hydrogen bond  $\text{X-H} \cdots \text{Y}$  will have three frequencies associated with it (*cf.* Fig. 2). So far only  $\nu_3$ , *i.e.*, the modified X-H frequency, has been treated; far less is known of the other two.  $\nu_1$  has been identified with the 160–175  $\text{cm}^{-1}$

<sup>8</sup> *J. Chem. Physics*, 1942, **10**, 607.

<sup>9</sup> *Trans. Faraday Soc.*, 1940, **36**, 889.

band observed in the Raman spectrum of water<sup>10</sup> which was also measured as an infra-red absorption by C. H. Cartwright.<sup>11</sup> Cartwright and J. Errera<sup>12</sup> confirmed the assignment when they showed the disappearance of this absorption in dioxan solutions of water. Similarly, liquid formic acid shows a broad Raman band centred near 180 cm.<sup>-1</sup>. Acceptance of this value for the  $\nu_1$  frequency would give a force constant for the hydrogen bond (XH . . . Y) of about  $2.7 \times 10^4$  dynes per cm. This is near the value estimated by Halford<sup>89</sup> from thermodynamic data, and is to be compared with values of the order  $6 \times 10^5$  dynes per cm. for normal covalent X-H bonds.

The bending vibration  $\nu_2$  will clearly be related to the angular deformation of the X-H bond. The latter vibration gives rise to the  $6\mu$  absorption in H<sub>2</sub>O. Whilst association with another molecule will stretch the X-H bond and so cause a decrease in the valence frequency, it will tend to tie the hydrogen more firmly in its angular orientation. Accordingly, the deformation frequency can be expected to rise on association: this agrees with the observation that the  $6\mu$  band moves to shorter wave-lengths on going from vapour to liquid water.<sup>13</sup> Similarly, in phenol vapour the 1175 cm.<sup>-1</sup> absorption is assigned to  $\delta(\text{OH})$  of the monomer, and this becomes 1210 cm.<sup>-1</sup> in the liquid, *i.e.*,  $\nu_2$  of O-H . . . O.<sup>14</sup> Unfortunately, it is only in a few cases of molecules forming hydrogen bonds that these deformation frequencies have been unambiguously identified.

*Raman Spectra.*—In discussing the interpretation of infra-red observations on hydrogen bonds, frequent reference has been made to similar or additional indications provided by Raman spectra. The results obtained by the latter means are in the same sense as those of infra-red spectra. However, the more polar an X-H bond is, with the less intensity will its vibrations appear in the Raman spectrum. Again, when a single sharp frequency is replaced by a broad band the latter may not be readily detected on the photographic plate. For the further reason that Raman spectra are not easily recorded at low concentrations—*i.e.*, in the vapour or dilute solution—the indications of molecular interactions are not usually so clear-cut as from the infra-red. However, in addition to those observations already quoted, special mention should be made of the study of carboxylic acid association by P. Koteswaram,<sup>15</sup> the examination of hydrogen bonds in amides by A. L. S. Rao,<sup>16</sup> and similar cases, including esters in alcoholic solvents, by G. V. L. N. Murty and T. R. Seshadri.<sup>17</sup> These authors deal particularly with the changes in the carbonyl frequency near 1740 cm.<sup>-1</sup> which appears to provide the clearest criterion of hydrogen

<sup>10</sup> J. H. Hibben, "The Raman Effect", Reinhold Publ. Corp., New York, 1939, pp. 320 *et seq.*

<sup>11</sup> *Nature*, 1935, **136**, 181; *Physical Rev.*, 1936, **49**, 470.

<sup>12</sup> *Proc. Roy. Soc.*, 1936, *A*, **154**, 138.

<sup>13</sup> See especially 98(c).

<sup>14</sup> V. Williams, R. Hofstadter, and R. C. Herman, *J. Chem. Physics*, 1939, **7**, 802.

<sup>15</sup> *Z. Physik*, 1938, **110**, 118.

<sup>16</sup> *J. Indian Chem. Soc.*, 1941, **18**, 337.

<sup>17</sup> *Proc. Indian Acad. Sci.*, 1941, *A*, **15**, 154, 230, 238.



bonding in the Raman spectra of these molecules. The conditions in water have been the object of much special study: <sup>18</sup> the results, up to 1939, have been collected in Hibben's book.<sup>10</sup> In methylamine one of the ( $-\text{N} \begin{smallmatrix} \text{H} \\ \text{H} \end{smallmatrix}$ ) valency vibrations shifts by 98  $\text{cm}^{-1}$  to lower frequencies on passing from vapour to liquid, whilst a line arising from the deformation of the same group, found at 781  $\text{cm}^{-1}$  in the vapour, could not be located in the liquid.<sup>19</sup> This is substantial evidence of the interactions of these groups in the condensed phase.<sup>20</sup> The extensive Raman data for the various forms of oxalic acid have been supplemented and discussed by L. Kahovec, K. W. F. Kohlrausch, and J. Wagner.<sup>21</sup>

*Dielectric Polarisation.*—As the X-H groups most effective in hydrogen bonding are of a pronounced polar character, it is clear that dipole-moment studies will contribute significantly to our understanding of these systems. This has been recognised since the earliest days of dipole-moment measurements and much evidence has been accumulated on molecular polarisation and its variation with molecular association. It is important in this respect to emphasise, as Pauling has done,<sup>22</sup> that the interpretation of hydrogen bonding in terms of the dipolar character of X-H or as resulting from the ionic form  $\bar{\text{X}}-\overset{+}{\text{H}}$  of the same bond are physically equivalent, for the latter is merely the principal electronic distribution which gives rise to the dipole moment. At short distances the influence of the latter must be calculated from the actual charge distribution—*i.e.*, from the resolved dipole moment corrected for the distortion produced by the interaction.

One of the striking consequences of "hydrogen bonding" is the abnormally large dielectric constant of liquids such as  $\text{CH}_3\cdot\text{OH}$ ,  $\text{H}_2\text{O}$ ,  $\text{HF}$ , and  $\text{HCN}$  compared with those of other molecular species whose individual moments are of the same order of magnitude. This is a result of the local structure maintained by the pronounced interaction of the X-H dipole with its neighbours. On the basis of a general treatment outlined by L. Onsager<sup>23</sup> and extended by J. G. Kirkwood,<sup>24</sup> G. Oster and Kirkwood<sup>25</sup> have been able to calculate the dielectric constants of liquids of this type with reasonable success. Apart from its immediate interest, the significance of such a treatment is that it provides a physically sound approach to the quantitative evaluation of medium (or solvent) effects.

<sup>18</sup> R. Rao, *Proc. Roy. Soc.*, 1931, *A*, **131**, 489; 1934, *A*, **145**, 489; *Phil. Mag.*, 1934, **17**, 113; C. S. S. Rao, *Proc. Roy. Soc.*, 1935, *A*, **151**, 167; R. Ananthakrishnan, *Proc. Indian Acad. Sci.*, 1935, *A*, **2**, 291; *ibid.*, 1936, *A*, **3**, 201; P. C. Cross, J. Burnham, and P. A. Leighton, *J. Amer. Chem. Soc.*, 1937, **59**, 1134.

<sup>19</sup> J. S. Kirby-Smith and L. G. Bonner, *J. Chem. Physics*, 1939, **7**, 880.

<sup>20</sup> For the general influence of intermolecular fields on Raman frequencies, see R. Mecke and O. Vierling, *Z. Physik*, 1935, **96**, 559; and 91(c).

<sup>21</sup> *Z. physikal. Chem.*, 1941, *B*, **49**, 145.

<sup>22</sup> See 39(a), p. 268.

<sup>23</sup> *J. Amer. Chem. Soc.*, 1936, **58**, 1486.

<sup>24</sup> *J. Chem. Physics*, 1939, **7**, 911.

<sup>25</sup> *Ibid.*, 1943, **11**, 175.

Kirkwood's analysis leads to the following equation for the dielectric constant of a liquid ( $\epsilon$ )

$$\frac{(\epsilon - 1)(2\epsilon + 1)}{9\epsilon} \cdot V = \frac{4\pi N}{3} \left[ \alpha + \frac{g\mu^2}{3kT} \right]$$

where  $V$  = molar volume of the liquid,  $\alpha$  = optical polarisability,  $\mu$  = molecular dipole moment in the liquid. The factor  $g$  is the term essential to the interpretation of the "associated", *i.e.*, thermodynamically abnormal, liquids :

$$g = [1 + z(\cos \gamma)_{\text{Av.}}]$$

where  $z$  = the average number of neighbours beyond which the dielectric constant applicable to a given molecule is effectively equal to the macroscopic value for the medium, *i.e.*, the number of neighbours of a given molecule for which appreciable correlation of orientation exists; and  $(\cos \gamma)_{\text{Av.}}$  is the average value of the cosine of the angle between the given dipole and one of these neighbours. The divergence of  $g$  from unity measures the influence of a molecule on the free rotation of its neighbours : it has thus been proffered as an appropriate measure, on the structural side, for the abnormality of a liquid. However, it depends both on the number of neighbours and on their relative orientations : a fixed antiparallel association of two dipoles gives  $g = 0$ , whilst parallel dimers correspond to  $g = 2$ . As is well known,  $\mu$  the dipole moment in the liquid differs from the value  $\mu_0$  for the gas owing to the induction of an additional moment by the dipole field of the neighbours. This may be expressed as

$$\mu_0 = \mu \left[ 1 - \frac{2\alpha z \cos^2 \frac{1}{2}\theta}{r^3} \right]$$

where  $r$  is the distance and  $\theta$  the angle between neighbours. The values of  $z$  are determined experimentally from the area under the appropriate peaks in the radial distribution function found for the liquid from its X-ray diffraction. Using the experimental values of  $z$  and  $r$  obtained by that means, Oster and Kirkwood, assuming that only neighbours in the first co-ordination shell contributed to  $z$ , obtained the following values for liquid water ( $\mu_0 = 1.81$  D.) :

	Temp.	0°.	25°.	62°.	83°.
$\epsilon$ (calc.)	.....	84.2	78.2	72.5	67.5
$\epsilon$ (obs.)	.....	88.0	78.5	66.1	59.9

Somewhat poorer agreement was attained ( $\sim 15\%$ ) for the alcohols from similar experimental data : it was assumed that the values  $z = 2$  (see p. 13) in these cases indicated association into chains and that there was no correlation of orientation between the chains. This last assumption was almost certainly the main source of the divergences found.<sup>26</sup>

G. Oster<sup>27</sup> has used the same equations to give a quantitative account of the dielectric constants of dilute aqueous solutions in a large number

<sup>26</sup> R. H. Cole, *J. Chem. Physics*, 1941, 9, 251.

<sup>27</sup> *J. Amer. Chem. Soc.*, 1946, 68, 2036.

of cases where "hydrogen bonds" with the solvent are certain to occur. It has been found experimentally that the dielectric constants of such solutions vary linearly (having slope  $\delta$ ) with concentration :

Solute.	MeOH.	EtOH.	<i>n</i> -PrOH.	Bu <sup>o</sup> OH.	COMe <sub>2</sub> .	Et <sub>2</sub> O.	NH <sub>2</sub> Ph.	C <sub>6</sub> H <sub>5</sub> N.
- $\delta$ (calc.) ...	1.79	3.11	4.22	6.26	4.13	7.6	6.4	5.3
- $\delta$ (obs.) ...	1.4	2.6	4.0	6.3	3.2	7.1	7.6	4.2

The agreement is only that of a first approximation, but it suggests that with further refinement this purely electrostatic treatment would give a satisfactory quantitative account of the observations.

Extensive measurements of dielectric polarisation in relation to the association of alcohols and carboxylic acids have been made over a number of years by K. L. Wolf and his collaborators. These have been summarised in part.<sup>28</sup> The treatment of the data collected has almost invariably been of a descriptive nature—in terms of the varying degrees of solute association—a process sometimes involving arbitrary assumptions as to the polarity of the associated molecules. However, the results, in conjunction with the parallel measurements of heats of dilution,<sup>29</sup> probably suffice to establish the different classes of molecular association involving hydrogen bonds.<sup>30</sup> The first group of solutes includes those such as the higher fatty acids in benzene and *tert*-butyl alcohol in *cyclohexane* for which there is equilibrium between monomer and only one associated species, usually the dimer. The second group is that for which a definite limited number of associated species can be recognised. It is of some general interest that the lower fatty acids are included here on the basis of evidence suggesting association beyond the dimer stage.<sup>31</sup> The third group is of molecules giving rise to a whole series of increasingly complex associates, the equilibrium constants for the successive stages often being of approximately the same value. Phenols and *n*-alcohols belong to this last class. Kempter and Mecke's quantitative analysis of phenol association in carbon tetrachloride suggests that in 6*M*-solution 25% of the solute is present as aggregates of ten or more molecules. Wolf, Dunken, and Merkel give similar data for ethyl alcohol in *cyclohexane*. H. Harms<sup>32</sup> has emphasised that the character of the association complex can change with solvent. The lower alcohols give complexes in benzene of enhanced polarity (possibly parallel dipoles): in *cyclohexane* the polarity is reduced on association (as for antiparallel dipoles). It is suggested that this variation results from the different small energies of solvent interaction. Calculations indicate that the energy difference between parallel and antiparallel association of the O-H dipoles may not be large.

<sup>28</sup> H. Dunken, K. Judenberg, and K. L. Wolf, *Z. physikal. Chem.*, 1941, B, **49**, 43; and further references there.

<sup>29</sup> Summary: K. L. Wolf, H. Dunken, and K. Merkel, *ibid.*, 1940, B, **46**, 287.

<sup>30</sup> See also, e.g., C. R. Bury and H. O. Jenkins, *J.*, 1934, 688.

<sup>31</sup> See also F. H. MacDougall, ref. (12).

<sup>32</sup> *Z. physikal. Chem.*, 1939, B, **43**, 257.

Considerations of this nature are also advocated by Hückel.<sup>33</sup> Benzyl alcohol in various solvents shows a concentration change of molecular polarisation which is markedly influenced by temperature; and the different behaviour of primary and secondary alcohols—especially when the hydroxyl group in the latter is sterically protected—is interpreted along the above lines. A more general treatment of the association process in terms of “swarm-formation” has been developed by A. Hartmann.<sup>34</sup> Amongst a number of dipole measurements in the gas phase<sup>35</sup> those for *o*-chlorophenol are best interpreted on the basis of an equilibrium between the *cis*- and *trans*-forms, with an energy difference between the isomers of  $1200 \pm 500$  cal. per g.-mol. The uncertainty in the energy value derived in this way arises principally from the possible error in the dipole moment ascribed to the *cis*-isomer.

Recently, Rodebush and his co-workers have contributed to polarisation studies of solutes forming hydrogen bonds. The first paper<sup>36</sup> emphasises the limitations of the Debye-Clausius-Mosotti treatment of dilute solutions. The earlier qualitative interpretations of “abnormalities” in dielectric polarisation results must certainly be reviewed in the light of these limitations which are now well established and beyond which the Onsager-Kirkwood relations (amongst others) are a distinct advance. A second paper<sup>37</sup> deals with the alcohols in carbon tetrachloride solutions, but although the results extend to mole-fractions  $\sim 0.005$  and cover the temperature range  $-10^\circ$  to  $+50^\circ$ , they do not allow of an unequivocal interpretation being advanced. A special mechanism suggested to account for the different dielectric behaviour of phenol solutions is not convincing.<sup>38</sup> An especially careful dielectric study of the carboxylic acids in organic solvents has been published by Hobbs, Gross, and their co-workers.<sup>39</sup> The measurements cover the solute mole-fraction range  $10^{-5}$  to  $10^{-2}$ , over which the monomer  $\rightleftharpoons$  dimer equilibrium shifts from one side to the other. Evaluation of the parameters by a process of successive approximations allows of the data being quantitatively reproduced in terms of the equilibrium constant and the apparent moments of the monomer and dimer molecules in solution. These “apparent moments” are in sensible agreement with the gas values. The most significant item is the molar polarisation of the dimer; this is some 16–25 c.c. larger than would be expected for the symmetrical dimer structure—*i.e.*, it corresponds to a moment for the dimer of about 1.0 D. The results for substituted benzoic acids—in particular for *p*-fluorobenzoic acid—make it clear, however, that the dimer has a symmetrical dipole structure and the “apparent moment” of the latter

<sup>33</sup> W. Hückel and I. Schneider, *Z. physikal. Chem.*, 1940, B, **47**, 227; W. Hückel and U. Wenzke, *ibid.*, 1942, B, **51**, 144.

<sup>34</sup> *Ibid.*, 1942, B, **51**, 309; 1942, B, **53**, 37, 49, 54.

<sup>35</sup> R. Linke, *ibid.*, 1940, B, **46**, 261.

<sup>36</sup> W. H. Rodebush and C. R. Eddy, *J. Chem. Physics*, 1940, **8**, 424.

<sup>37</sup> W. H. Rodebush, C. R. Eddy, and L. D. Eubank, *ibid.*, p. 889.

<sup>38</sup> W. H. Rodebush and C. Kretschmer, *ibid.*, 1941, **9**, 284.

<sup>39</sup> H. A. Pohl, M. E. Hobbs, and P. M. Gross, *ibid.*, p. 408; A. A. Maryott, M. E. Hobbs, and P. M. Gross, *ibid.*, p. 415.

is taken to indicate an atomic polarisation of about 20 c.c. This agrees with the earlier conclusion of I. E. Coop, N. R. Davidson, and L. E. Sutton<sup>40</sup> based on formic acid vapour measurements. In view of the probable association beyond the dimer in the case of some carboxylic acids the presence of additional species should be considered as a possible source of the large atomic polarisation now allotted to the dimer.

The difficulties encountered by ascribing "hydrogen bonding" to electrostatic effects are well known. The weak association of esters, ethers, and alkyl amides despite the presence in their structures of dipoles comparable with the hydroxyl, and the great difference in the water solubilities of propyl chloride and propyl alcohol are typical of the facts which have been deemed to require a special mechanism in the "hydrogen bond". Only quantitative evaluation of the permissible interaction energies in individual cases can decide this point, but it is immediately clear that in the first instances mentioned the dipoles are frequently "buried" within the molecules and may well be ineffective through a form of steric hindrance. As would be expected on these grounds, steric factors can greatly diminish "hydrogen bonding":<sup>41</sup> this is even true of the solid state where, given suitable steric protection, the hydroxyl group remains essentially "free".<sup>42</sup> Thus the unique features about a dipole involving a hydrogen atom arise from the univalency of the latter and its small size. Both factors contribute in an important way to its behaviour. It has often been emphasised that the concentration of effective charge will be much greater on the hydrogen than on any other member of a comparable dipole. The result may be roughly illustrated by a comparison of the attractive fields for the C-Cl and O-H dipoles, both of them being taken to be of moment 1.5 D. In the absence of any special interaction, an adjacent molecule would approach (in the line of the dipole) to the limit set by the "van der Waals radius" of the chlorine and hydrogen respectively, i.e., to 1.8 and 1.2 Å. from the corresponding atomic centres.<sup>42</sup> At these positions the net electrostatic dipole field ( $\Sigma e/r^2$ ) for the two cases are in the ratio 0.20 : 0.75, i.e., the hydroxyl dipole has approximately four times the effective strength of the C-Cl. Such considerations might well account for the differences between propyl chloride and propyl alcohol.

*Miscellaneous Studies.*—Only a bare mention can be made of some further physical studies of hydrogen bonds. The bulk of thermochemical data is not sufficiently precise to detect the small changes that may arise from the weaker forms of hydrogen bonds.<sup>43</sup> The relation of diamagnetic susceptibility and hydrogen bonding has been examined,<sup>44</sup> and polaro-

<sup>40</sup> *J. Chem. Physics*, 1938, **6**, 905.

<sup>41</sup> See, e.g., A. E. Alexander, *Proc. Roy. Soc.*, 1942, *A*, **179**, 470; also Hückel and Schneider, ref. (33).

<sup>42</sup> The data used are taken from L. Pauling, *op. cit.* (39a).

<sup>43</sup> A. Sherman, *J. Physical Chem.*, 1937, **41**, 117.

<sup>44</sup> W. R. Angus and W. K. Hill, *Trans. Faraday Soc.*, 1940, **36**, 923; H. S. Venkataraniiah, *Chem. Abs.*, 1942, **36**, 6385; F. v. Rautenfeld and E. Streurer, *Z. physikal. Chem.*, 1941, *B*, **51**, 39.

graphic studies have been made.<sup>45</sup> A systematic account of the contribution of hydrogen bonds to the stability of condensed monolayers has been given.<sup>46</sup>

*Conclusions.*—The present review appears to indicate sensibly consistent progress in our understanding of the hydrogen bond. Viewed as an example of molecular (or group) interaction, the principal energy item is of an electrostatic nature, the contributions of resonance effects in this direction being usually small, if not entirely negligible. As early as 1933, G. Briegleb<sup>47</sup> had indicated that hydrogen bonds were found in cases where, for the items that could be estimated, dipole forces were the predominant attractive term at the equilibrium distance of approach. However, in questions of structural isomerism<sup>2</sup> resonance may yet play an important rôle. Thus, Ketelaar's estimate being accepted of the ground level stabilisation in  $(F-H \dots F)^-$ , i.e.,  $\frac{1}{2} \Delta v \sim 10 \text{ cm.}^{-1}$  (which should, of course, give rise to a strong absorption at  $\lambda \sim 0.5 \text{ mm.}$ ), this small value means a hydrogen oscillation between the fluorines of frequency in the region of  $6 \times 10^{11}$  per sec. Thus, even very much smaller resonance energy terms (giving a proportionately reduced value for this frequency) would still provide an oscillation far too rapid to allow of separating what might be isomeric molecules.

Only in a very broad sense is the phenomenon of "hydrogen bonding" dependent upon the same factors as control the generality of chemical bonds: it clearly has more in common with the forces present in "van der Waals molecules" than with any other type. In this respect the term "hydrogen bond" is an unfortunate one, the more so if it is taken to suggest the operation of special factors nowhere, or rarely, found in its absence. The name "hydrogen bridge" favoured by Huggins,<sup>4a</sup> and frequently used in the American literature, has much to commend it. The frequency shifts recorded in the infra-red show clearly that the covalent bond to the hydrogen is extended only by a matter of some few per cent. in the interaction. This leads to a corresponding increase in the X-H bond moment; alternatively, the change may be regarded as an increase in the ionic character of the bond. The change in this direction is further indicated by the enhanced intensity of the infra-red absorptions on hydrogen bonding. However, the evidence seems quite definite that the X-H bond is not greatly disturbed. The success of the calculations accounting for the bond energies and the dielectric properties in terms of the normal dipole values, etc., whilst not complete, is sufficiently good to leave no room for uncertainty as to the principal factors controlling the phenomenon.

On the basis of theoretical ideas that still remain valid Bernal and Megaw used the terms "hydrogen bond" and "hydroxyl bond" to distinguish what

<sup>45</sup> M. J. Astle and W. V. M. Connell, *J. Amer. Chem. Soc.*, 1943, **65**, 35; M. J. Astle and W. F. Croper, *ibid.*, p. 2395; M. J. Astle and S. P. Stephenson, *ibid.*, p. 2399.

<sup>46</sup> A. E. Alexander, ref. (41); *Proc. Roy. Soc.*, 1942, *A*, **179**, 486.

<sup>47</sup> *Z. physikal. Chem.*, 1933, *B*, **23**, 105.

would be the extreme types in a continuous range of interactions. The practical criteria suggested for recognising the former (the symmetrical location, or mobility, of the hydrogen, *e.g.*, in  $\text{KH}_2\text{PO}_4$ , and disappearance of the infra-red absorption) lead to the conclusion that no true "hydrogen bond" is at present known. To the Reporter it appears that little of established physical fact is indicated by the retention of the dual classification. It may be, however, that in some few ionic solids the forces normally controlling the interaction assume an extreme form and are there associated with novel co-operative phenomena.

M. D.

## 2. THE MOLECULAR WEIGHT AND DIMENSIONS OF MACROMOLECULES IN SOLUTION.

The construction and use of the first ultracentrifuge by Svedberg and his collaborators in 1925 gave a great stimulus to the precise investigation of colloidal systems, and the subsequent years have seen a rapid development in our knowledge of particle weight and dimensions in such systems. In addition to the techniques involving the ultracentrifuge, other new methods have been evolved and existing ones improved and extended. Here we are concerned, first, with reviewing the available methods (Part I), and secondly with discussing their application in representative systems of macromolecules (Part II).

### *Part I. Experimental Methods.*

It is convenient to subdivide the experimental methods to be described here into: (1) the thermodynamic or equilibrium methods; (2) the dynamic methods; (3) miscellaneous methods (including optical, X-ray, and analytical methods).

In the first group, the physical measurements, upon which the calculations of molecular weight are based, refer to a system in equilibrium whose properties may therefore be described in terms of thermodynamics. This thermodynamic basis constitutes one of the important properties of the group of methods; at the same time, however, it imposes limitations both in the use of experimental data to derive molecular weight and in obtaining further information on molecular size. The nature of these limitations will shortly become clear.

The second group, the dynamic methods, possesses no thermodynamic basis, being concerned not with equilibrium states but chiefly with the rate at which certain types of motion are executed by colloidal particles or macromolecules. Fundamentally these methods are not so sound as the thermodynamic ones, but they do offer the possibility of obtaining, not only molecular weight, but also certain information on molecular shape and dimensions.

In the third group we consider the methods based upon the diffraction of X-rays, the scattering of visible light and certain analytical methods which have proved useful.

Difficulties and uncertainties have been and still are encountered in the

use of these methods and it is essential, especially in the earlier stages, that each should, wherever possible, be checked against others, preferably of a different group. By such counter-checking a body of sound information on macromolecular systems, with which we shall be concerned in Part II of this Report, is developing.

(1) *The Thermodynamic or Equilibrium Methods.—Osmotic pressure.* Of the colligative methods, which are so largely used in investigating low-molecular-weight substances in solution, only that of osmotic pressure has, so far, been widely used for determining the molecular weights of macromolecules, the others being experimentally too insensitive and prone to excessive interference by low-molecular-weight impurities. The osmotic method, in spite of its extra sensitivity, is not sensitive enough for determining the highest polymer molecular weights; nor is it always possible to avoid the osmotic effects of low-molecular-weight impurities, though these may be allowed for.

The basic equation of osmotic pressure, derived thermodynamically, may be written :

$$\pi = \frac{RT}{V_1} \ln \frac{p_1^0}{p_1} \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where  $\pi$  is the osmotic pressure,  $V_1$  the partial molar volume of the solvent, and  $p_1^0$  and  $p_1$  the solvent vapour pressure over pure solvent and solution, respectively. This equation is universally valid over the whole concentration range. Other equations, and especially that of van't Hoff, *viz.*,

$$\pi = cRT/M \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where  $c$  is the concentration in g./litre of solute of molecular weight  $M$ , have a much more limited application. To derive (2) from (1) it is necessary to assume (i) that the solution is very dilute and (ii) that Raoult's law is obeyed. For low-molecular-weight solutes in dilute solution ( $< 3\%$ ), Raoult's law holds in general, but for solutions of macromolecules at comparable concentration, serious deviations from the ideal behaviour expressed by the law are encountered. In the case of the linear chain-like polymers, these deviations, which are particularly serious, are largely due to the abnormally high entropy of mixing of the polymer and solvent. It has been calculated<sup>1</sup> on this basis by statistical methods that the osmotic pressure-concentration relation should be of the type

$$\pi/c = RT/M + Kc \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$K$  being a constant depending upon the particular polymer-solvent system. If an equation of this form, for which experiment has provided considerable evidence (see p. 57), be accepted, then in place of the simpler expression for molecular weight derived from the van't Hoff law ( $M = cRT/\pi$ ), we have

$$M = RT \lim_{c \rightarrow 0} c/\pi \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Usually, experimental measurements of osmotic pressure are utilised as a plot of  $\pi/c$  against  $c$ , the intercept on the  $\pi/c$  axis giving  $\lim_{c \rightarrow 0} \pi/c$  and the slope

<sup>1</sup> G. Gee, *Ann. Reports*, 1942, **39**, 7.



of the curve the value of  $K$ , a measure of the deviations from ideal behaviour. Clearly, the accurate determination of molecular weights depends upon the accuracy of the extrapolation yielding  $Lt.\pi/c$ , and therefore upon the use of low concentration measurements and the choice, where possible, of systems for which  $K$  is zero or small. In certain types of system (*e.g.*, linear polymers in good solvents) high values of  $K$  cannot be avoided; the efforts of recent years to reduce experimental error and to increase the accuracy of low concentration measurements are therefore of great importance in such systems. In other systems (*e.g.*, corpuscular proteins) the difficulties of extrapolation may not be so great but osmotic pressure readings at low solute concentration (1% and less) are still essential.

In the experimental measurement of osmotic pressure, different techniques have been developed appropriate to aqueous and organic solvents. In the simplest and most frequently used osmometer for aqueous solutions, based upon that of G. S. Adair,<sup>2</sup> the solution is contained in a thimble-shaped membrane of cellulose nitrate, mounted securely upon the capillary observation tube and immersed in the solvent medium. The use of capillary tubing for observing osmotic pressure-heads makes possible the more rapid attainment of equilibrium, but also necessitates a correction, of rather uncertain magnitude, for the capillary rise. Other workers<sup>3, 4, 5, 6</sup> have attempted to avoid this difficulty by suitable osmometer design; the use of an organic solvent (usually toluene) with its smaller and more reproducible capillary rise, by the last three workers, deserves mention in this respect. Some of the osmometers suggested require the use of rather large volumes of liquid and are therefore extremely sensitive to temperature change: it is doubtful in such cases if improved results are obtained. Further developments in this field are, however, to be expected.

In the case of charged polymer solutions, containing other diffusible ions, Donnan effects are eliminated where possible by the use of suitably large salt concentrations, or are allowed for by the calculation of ion pressure differences from measurements of membrane potentials.<sup>7</sup>

Measurements of osmotic pressure in organic solvents differ from those in aqueous solvents in the reduced magnitude of capillary corrections and in the usual absence of Donnan effects. Deviations from ideal behaviour, which as we have seen are very serious in the case of linear polymers, may, however, more than compensate for such simplifications. Special forms of osmometer, usually constructed in non-corrosive metal, utilising large membrane areas and small liquid volumes, and allowing rapid equilibration, have therefore been constructed;<sup>8</sup> the osmotic head, observed as the difference in the liquid levels in the two identical pieces of precision capillary tubing, being

<sup>2</sup> G. S. Adair, *Proc. Roy. Soc.*, 1925, **A**, **108**, 627.

<sup>3</sup> H. B. Oakley, *Trans. Faraday Soc.*, 1935, **31**, 136.

<sup>4</sup> J. Bourdillon, *J. Biol. Chem.*, 1939, **120**, 63.

<sup>5</sup> H. B. Bull, "Physical Biochemistry", Wiley, 1943, p. 300.

<sup>6</sup> G. S. Adair, private communication. <sup>7</sup> *Idem*, *Proc. Roy. Soc.*, 1929, **A**, **126**, 16.

<sup>8</sup> See, *e.g.*, R. M. Fuoss and D. J. Mead, *J. Physical Chem.*, 1943, **47**, 59.

reached in a time usually less than one hour. To achieve such rapid equilibration even with large membrane areas, specially permeable membranes of partly denitrated cellulose nitrate <sup>8</sup> or bacterial cellulose secretion <sup>9</sup> have been used. More rapid estimates of the final equilibrium osmotic head may also be made by the so-called "dynamic" method in which the rate of movement of the meniscus on the solvent side of the cell (with constant solution height) is plotted as a function of the meniscus position, and extrapolated to zero rate. This method of observing osmotic pressures, which clearly retains its thermodynamic basis, is not to be confused with the group of dynamic methods which, as we have indicated, possess no such basis.

Two recent developments are to be reported : (i) the construction of the osmotic balance; (ii) the use of light scattering measurements. In the osmotic balance <sup>10</sup> liquid flow through a membrane is followed by weighing on an analytical balance rather than by measurement of the height of a liquid column. Clearly, the method has considerable possibilities, but there are severe experimental requirements (especially the rigid temperature control of the balance for long periods) and though very low concentration measurements of osmotic pressure have been reported, it is yet too early to decide whether the method is superior to the best forms of the normal osmometer.

The light scattered by a solution in excess of that scattered from the pure solvent is due to the concentration fluctuations occurring in the solution, and since the extent of such fluctuations is clearly dependent on the free-energy changes involved, a connection between light scattering and the thermodynamic properties of a solution is involved.

Following von Smoluckowski and Einstein, and assuming an osmotic pressure concentration relation of the type of equation (3), the following equation connecting the turbidity  $\tau$  (or fractional decrease, due to scattering, in the incident intensity on traversing 1 cm. of solution) and molecular weight was obtained : <sup>11, 12</sup>

$$\frac{32\pi^3 n^2 (\partial n / \partial c)^2}{3\lambda^4 N} \cdot \frac{c}{\tau} = H \cdot \frac{c}{\tau} = \frac{1}{M} - \frac{2Kc}{RT} \quad . \quad . \quad . \quad (5)$$

where  $n$  is the refractive index of the solution,  $\lambda$  is the wave-length of the incident light, and  $N$  is Avogadro's number. R. S. Stein and P. Doty <sup>13</sup> have already reported the use of this equation in determining the molecular weight of certain cellulose acetate fractions.

Other aspects of light-scattering measurements will be discussed under group (3) methods.

Although higher accuracy has been claimed, in general, osmotic pressure heads are accurate only to *ca.*  $\pm 0.05$  cm. The osmotic pressure of a 1%

<sup>9</sup> H. W. Melville, C. R. Masson, J. Cruickshank, and R. F. Menzies, *Nature*, 1946, 157, 74.

<sup>10</sup> I. Jullander, *Arkiv Kemi. Min. Geol.*, 1945, 21A, No. 8.

<sup>11</sup> P. Debye, *J. Appl. Physics*, 1944, 15, 338.

<sup>12</sup> P. Doty, B. H. Zimm, and H. Mark, *J. Chem. Physics*, 1945, 13, 159.

<sup>13</sup> *J. Amer. Chem. Soc.*, 1946, 68, 159.

solution of a macromolecular substance of molecular weight 70,000 being taken to be 3 cm. of water, the error involved in measuring this head is therefore  $\pm 1.6\%$ . If lower concentrations of solute are necessitated by high deviations, or where the solute is of higher molecular weight, pressure observations and the molecular-weight calculations resulting are correspondingly less accurate.

Clearly, the osmotic pressure method alone can give no information on polydispersity, the mean molecular weight obtained in such systems being a number average ( $M_N$ ) defined by

$$M_N = \Sigma n_i M_i / \Sigma n_i \quad . \quad . \quad . \quad . \quad . \quad (6)$$

where  $n_i$  is the number of molecules of molecular weight  $M_i$ . It should be noted that turbidity measurements differ in that they yield the weight average defined by

$$M_w = \Sigma n_i M_i^2 / \Sigma n_i M_i \quad . \quad . \quad . \quad . \quad . \quad (7)$$

*Sedimentation Equilibrium.*—The fall-off in the density of the earth's atmosphere with height, and the concentration distribution in colloidal solutions used by Perrin to determine Avogadro's number, are well-known examples of the influence of the earth's gravitational field. In the sedimentation equilibrium method of determining molecular weight, a centrifugal field ( $\approx 10,000$  g.) takes the place of gravity, and the concentration distribution set up in a colloidal solution may be used to calculate the particle or molecular weight of the colloidal solute.<sup>14</sup> The solution, in a cell with transparent windows, is spun without vibration at high speeds ( $\approx 15,000$  r.p.m.) until equilibrium is set up, the concentration distribution being examined optically during this process.

At equilibrium, the system may be treated thermodynamically, and for ideal behaviour it may be shown<sup>15</sup> that

$$M = \frac{2RT \ln c_2/c_1}{(1 - V\rho)\omega^2(x_2 - x_1)} \quad . \quad . \quad . \quad . \quad . \quad (8)$$

where  $c_1$  and  $c_2$  are the solute concentrations at distances  $x_1$  and  $x_2$  from the axis of rotation,  $V$  is the partial specific volume of the solute,  $\rho$  the density of the solution, and  $\omega$  the angular velocity of rotation. The same equation may also be obtained by the kinetic method of equating at any point in the cell the flow of solute in the direction of the centrifugal field with the opposing flow due to diffusion (see pp. 38, 43).

Equation (8) being, however, dependent on ideal behaviour holds only under conditions for which other thermodynamic phenomena (*e.g.*, osmotic pressure) behave ideally. For low-molecular-weight solutes, or high-polymeric substances of nearly spherical molecular shape, it is therefore valid at low concentrations ( $\approx 1\%$ ). Under other conditions, the deviations from ideal behaviour must be considered quantitatively, and this may be done by

<sup>14</sup> T. Svedberg and K. O. Pedersen, "The Ultracentrifuge", Oxford, 1940; see also symposium on the ultracentrifuge, *Ann. N. Y. Acad. Sci.*, 1942, **43**, 176.

<sup>15</sup> See, *e.g.*, E. A. Guggenheim, "Modern Thermodynamics", Methuen, 1933, p. 153.

either the thermodynamic or the kinetic method. By the first method, the concentrations  $c_1$  and  $c_2$  are to be replaced by activities,  $c_1f_1$  and  $c_2f_2$ , and the determination of molecular weights depends therefore on a knowledge of the activity coefficient ( $f$ ) throughout the cell. This method has not yet been employed. In the second method, the effect of increasing concentration on the involved processes of sedimentation and diffusion is considered, when a modified expression is obtained in place of equation (8). N. Gralen,<sup>16</sup> assuming the sedimentation constant ( $s$ ) and diffusion coefficient ( $D$ ) to vary with concentration according to the expressions

$$s = s_0/(1 + kc) \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

and

$$D = D_0(1 + k_1c) \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

where  $k$  and  $k_1$  are constants, and  $s_0$  and  $D_0$  are the values of  $s$  and  $D$  at zero concentration, obtained the sedimentation equilibrium expression

$$M = \frac{2RT[\ln(c_2/c_1) + (k + k_1)(c_2 - c_1) + \frac{1}{2}kk_1(c_2^2 - c_1^2)]}{(1 - V\rho)\omega^2(x_2^2 - x_1^2)} \quad . \quad (11)$$

Clearly, where sedimentation constant and diffusion coefficient are independent of concentration, equation (11) reduces to the ideal expression. An example of the use of this corrected equation will be given in Part II.

Table I contains the sedimentation-equilibrium results for monodisperse carboxyhaemoglobin in an aqueous buffer medium;  $x_1$  and  $x_2$  are given in cm., and  $c_1$  and  $c_2$  in g./100 c.c. With increasing distance from the axis of rotation, the concentration of solute, whose density is greater than that of the solvent, increases continuously. Deviations from ideal behaviour are therefore expected to be more pronounced at the higher  $x$  values; the reduced molecular-weight values characteristic of such deviations are not, however, prominent in Table I, but the deviations are shown very prominently for the

TABLE I.

*Sedimentation equilibrium of carboxyhaemoglobin.*

(From T. Svedberg and R. Fahraeus.<sup>17</sup>)

Initial solute concn. = 1.0 g. per 100 c.c.;  $V = 0.749$ ;  $T = 293.3^\circ \text{K.}$ ; speed = 8708 r.p.m.; time of centrifuging = 39 hours.

$x_2$ .	$x_1$ .	$c_2$ .	$c_1$ .	$M$ .	$x_2$ .	$x_1$ .	$c_2$ .	$c_1$ .	$M$ .
4.61	4.56	1.220	1.061	71,300	4.36	4.31	0.639	0.564	60,990
4.56	4.51	1.061	0.930	67,670	4.31	4.26	0.564	0.496	76,570
4.51	4.46	0.930	0.832	58,330	4.26	4.21	0.496	0.437	69,420
4.46	4.41	0.832	0.732	67,220	4.21	4.16	0.437	0.388	66,400
4.41	4.36	0.732	0.639	72,950					

linear polymer, cellulose nitrate in amyl acetate, for which sedimentation equilibrium results are shown later in Table V.

For a dilute system containing two or more types of corpuscular macromolecule (*i.e.*, a paucidisperse system), each type may be considered to reach

<sup>16</sup> "Sedimentation and Diffusion Measurements on Cellulose and Cellulose Derivatives", *Diss.*, Uppsala, 1944, p. 76.

<sup>17</sup> *J. Amer. Chem. Soc.*, 1926, **48**, 430.

an equilibrium-concentration distribution independently, the higher-molecular-weight molecules being relatively more concentrated (for  $\rho_{\text{solute}} > \rho_{\text{solvent}}$ ) at the higher  $x$  values. The calculated molecular weights therefore increase with increasing  $x$  values (a drift opposite to that arising from imperfect behaviour) as is shown by Table II for unelectrodialysed egg-white.

TABLE II.

*Sedimentation equilibrium of unelectrodialysed egg albumin.*(From T. Svedberg and J. B. Nichols.<sup>18</sup>)

Initial solute concn. = 0.78 g. per 100 c.c.;  $V = 0.749$ ;  $T = 288^\circ \text{K.}$ ; Speed = 10,550 r.p.m.

$x_2$	$x_1$	$c_2$	$c_1$	$M$
4.680	4.630	1.0455	0.9149	45,300
4.630	4.581	0.9149	0.8077	42,750
4.581	4.531	0.8077	0.7128	43,300
4.531	4.482	0.7128	0.6386	38,500
4.482	4.432	0.6386	0.5754	36,950
4.432	4.382	0.5754	0.5196	36,550
4.382	4.333	0.5196	0.4708	35,700
4.333	4.283	0.4708	0.4277	35,200
4.283	4.233	0.4277	0.3890	35,150

Some knowledge of the composition of the system may be obtained from such a variation in calculated molecular weight with  $x$  values, but the total concentration distribution is not very sensitive to small amounts of material and the information obtained is only of a qualitative character: especially so where imperfect behaviour is also present. It will later be shown that the sedimentation-velocity method gives much more clear-cut information for paucidisperse systems.

It should be noted that the molecular weight calculated at any  $x$  value of the cell is not any well-defined mean value, but if this is integrated over the cell, the value obtained may be either a weight average [equation (7)], or a "z" average value defined by

$$M_z = \Sigma n_i M_i^3 / \Sigma n_i M_i^2 \quad . \quad . \quad . \quad . \quad . \quad (12)$$

according to the method of calculating the results.

The high-molecular-weight limit of the method is set by slowness in establishing equilibrium for very high-molecular-weight solutes, the time required being inversely proportional to the diffusion coefficient of the solute. However, the method has been successfully employed for the almost spherical protein of bushy stunt virus<sup>19</sup> of molecular weight  $7.6 \times 10^6$ . For asymmetric polymers the limits are, however, much lower, as is indicated by Table V; and a high-molecular-weight limit considerably less than 100,000 for quantitative values would appear not unreasonable. By use of a very high-speed ultracentrifuge the method has been successfully employed to study sedimentation equilibrium in low-molecular-weight (e.g., NaCl, LiCl) electrolyte solutions.<sup>20</sup>

<sup>18</sup> *J. Amer. Chem. Soc.*, 1926, **48**, 3081.<sup>19</sup> A. S. McFarlane and R. A. Kekwick, *Biochem. J.*, 1938, **32**, 1607.<sup>20</sup> K. O. Pedersen, *Nature*, 1935, **135**, 304.

Summarising, we may say that the equilibrium methods provide useful means of determining molecular weights, the osmotic method being especially suitable for the lower-molecular-weight polymers ( $M < 200,000$ ) and the sedimentation equilibrium method for those of higher molecular weight, provided they are not too asymmetric in shape. In neither method is any information regarding molecular shape directly obtained, and this constitutes one of the disadvantages of the methods. The dynamic methods, which we shall now consider, are, however, more helpful in this respect.

(2) *The Dynamic Methods.*—As already indicated, the dynamic methods are concerned with the rate at which the dissolved molecules perform certain types of motion under the influence of applied forces of the appropriate kind. For given applied forces, the observed rates depend not only upon molecular weight (or volume) but also upon molecular shape. The methods therefore give some direct information on molecular shape, usually in the form of a frictional constant for the solute molecules, which is defined by an equation of the general form

$$\text{Force (or couple) per molecule} = \text{Frictional constant} \times \text{velocity} . \quad (13)$$

Experimental observation of the rate of a molecular process under a given applied force may therefore be used to determine the appropriate frictional constant. If the polymer molecules may be treated as spheres, ellipsoids, or other idealised forms, then the frictional constant may be expressed in terms of the molecular dimensions; knowledge of the frictional constant may therefore allow an evaluation of molecular dimensions. In such a procedure considerable difficulties of both a theoretical and a practical nature occur, some of which will be mentioned later. Here we mention one only, in view of its general nature, arising from the complex molecular configuration of certain substances. Quantitative information on molecular dimensions can as yet only be derived if the polymer may be treated on the basis of a regular, simple geometrical shape. For some molecules (*e.g.*, the corpuscular proteins), this assumption may be a justifiable approximation. For others (*e.g.*, flexible linear polymers), it is of doubtful validity, and information on their molecular dimensions derived from frictional ratios is therefore correspondingly doubtful.

We shall first consider those methods (translational diffusion, sedimentation velocity, electrophoresis) which involve the translational motion of the dissolved polymer molecules. In such cases equation (13) becomes

$$\text{Force} = f \cdot dx/dt \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

where  $f$  is the frictional constant per molecule and  $dx/dt$  is the translational velocity.

Although the different methods will be described separately, in view of the occurrence of the two (and possibly more) unknowns of molecular weight and shape, two independent experimental measurements are required in the evaluation of these quantities. Data from translational-diffusion and sedimentation-velocity measurements are thus very commonly combined, but other combinations are used as convenient.

*Translational diffusion.* When two parts of a system, containing a solute at different concentrations, are put into contact, the differences in chemical potential occurring over the boundary region give rise to a "force" by which solute passes from the higher to the lower concentration region until a uniform potential occurs throughout the system. The quantitative treatment of this translational diffusion of the solute is based upon Fick's laws which may be written

$$dm = -DA(dc/dx)dt \quad . \quad . \quad . \quad (15)$$

where  $dm$  is the quantity of solute diffusing in time  $dt$  across an area,  $A$ , at which the concentration gradient is  $dc/dx$ ,  $D$  being the diffusion coefficient, and

$$\partial c/\partial t = D(\partial^2 c/\partial x^2) \quad . \quad . \quad . \quad (16)$$

The solution of equation (16), the general differential equation of diffusion, depends upon the boundary conditions imposed. Putting for  $t = 0$ ,

$$\begin{aligned} x > 0, c &= 0, \\ x < 0, c &= c_0, \end{aligned}$$

$c_0$  being the initial concentration of solute, and assuming, (a) that the diffusion coefficient is independent of concentration and (b) that the two parts of the system extend to infinity in positive and negative directions, the solution of equation (16) is given by

$$c = \frac{c_0}{2} \left[ 1 - \frac{2}{\sqrt{\pi}} \int_0^y e^{-y^2} \cdot dy \right] \quad . \quad . \quad . \quad (17)$$

where  $y = x/2\sqrt{Dt}$ ,  $c$  being the concentration at time  $t$  at a distance  $x$  from the original boundary. The differential form of equation (17) may be written

$$\frac{dc}{dx} = \frac{c_0}{2\sqrt{\pi Dt}} \cdot e^{-x^2/4Dt} \quad . \quad . \quad . \quad (18)$$

The geometrical representation of equations (17) and (18) is given in Fig. 1 (a and b). With increasing values of  $t$ , the curve of equation (17) turns about

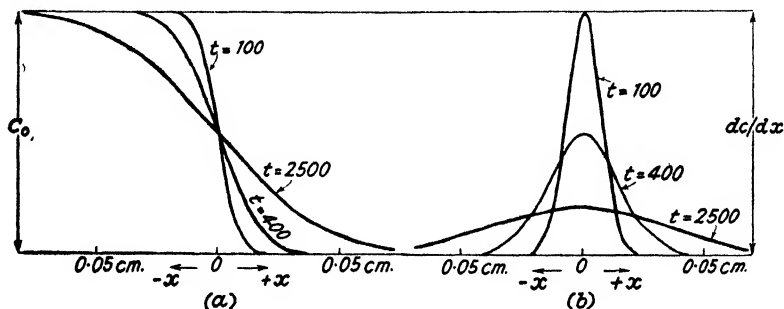


FIG. 1.

Calculated curves of concentration (a) and concentration gradient (b) against distance for free diffusion across a boundary:  $D = 2.5 \times 10^{-7}$  c.g.s. units. Time,  $t$ , in secs.

its point of inflection at  $x = 0$ , the "peak" of equation (18) simultaneously decreasing in height and becoming broader at constant area.

In the most important experimental method of measuring diffusion coefficients, a horizontal boundary between solution and solvent (or between two solutions of differing concentration) is formed in a vertical tube,<sup>21, 22, 23</sup> and its spreading with time is observed by light-absorption or refractive-index methods under conditions in which external disturbances (mechanical and thermal) are reduced to a minimum. The optical methods yield curves of  $dc/dx$  or  $c$  against  $x$  (usually the former) for different  $t$  values, and from these curves the diffusion coefficient may be calculated, by one of several methods which have been discussed in detail elsewhere.<sup>21, 22, 24</sup> Only in the case of monodisperse systems whose diffusion coefficients are independent of concentration do the different calculations agree: the divergencies in other cases are sometimes useful in giving information on polydispersity and diffusion coefficient-concentration dependencies.

Another method of measuring diffusion coefficients, known as the "porous-disc method,"<sup>25</sup> must be mentioned. The two fluids (solvent and solution or two solutions) are separated by a porous disc, in the pores of which a concentration gradient is established, and through which diffusion occurs. The liquids on both sides of the disc are kept homogeneous by various stirring devices,<sup>26</sup> and the diffusion is followed by analysis of the liquids. In obtaining absolute diffusion coefficients, calibration of the porous disc by solutes of known diffusion coefficients is necessary, but the difficulties involved have been such that it is now doubtful if, in general, the method can be as precise as that using a free boundary, though the suggestions of Hartley and Runnicles<sup>26</sup> and of Gordon *et al.*<sup>27</sup> represent a considerable advance on previous views. In considering further the experimental side of diffusion we shall concentrate chiefly on results from the free-boundary method.

Equation (18), which is valid only for a monodisperse ideal solute, is represented geometrically by a "Gaussian" curve which is symmetrical about the ordinate at  $x = 0$  (Fig. 1b). For a monodisperse solute in which the diffusion coefficient varies with the changing concentration over the boundary region, the  $dc/dx$ - $x$  curve is no longer symmetrical but is described as "skew". The  $dc/dx$ - $x$  curve for systems which are not monodisperse is given by the sum of the different curves for each different species, which, as we have seen, may or may not be symmetrical about the zero ordinate. In any case, however, the composite curve will not be of the ideal Gaussian form. Clearly therefore, a sensitive test of monodispersity and absence of imperfections is the degree of correspondence between experimental  $dc/dx$ - $x$  curves and the ideal Gaussian curves given by equation (18). Where the experimental and ideal curves correspond closely, the diffusion coefficient may be calculated by

<sup>21</sup> O. Lamm, *Nova Acta Reg. Soc. Scient. Upsala*, 1937, IV, 10, No. 6.

<sup>22</sup> H. Neurath, *Chem. Reviews*, 1942, **30**, 367.

<sup>23</sup> S. Claesson, *Nature*, 1946, **158**, 834.

<sup>24</sup> C. O. Beckmann and J. L. Rosenberg, *Ann. N.Y. Acad. Sci.*, 1945, **46**, 329.

<sup>25</sup> M. L. Anson and J. H. Northrop, *J. Gen. Physiol.*, 1929, **12**, 543.

<sup>26</sup> See, e.g., G. S. Hartley and D. F. Runnicles, *Proc. Roy. Soc.*, 1938, A, **168**, 401.

<sup>27</sup> W. A. James, E. A. Hollingshead, and A. R. Gordon, *J. Chem. Physics*, 1939, **7**, 89.



any suitable method, all methods giving identical values. If, however, polydispersity without imperfect behaviour is indicated by experimental curves which are symmetrical but nevertheless deviate from the ideal, then the different methods of calculation yield different average diffusion coefficients, *e.g.*, the weight average value is obtained by a statistical treatment of the curve. Comparison of the different types of average may, however, give a very useful indication of the degree of polydispersity.

Those systems in which diffusion coefficients are not independent of concentration cannot be rigidly treated by the methods already indicated, since one of the basic assumptions involved in solving the general differential equation of diffusion was the constancy of diffusion coefficients. In such cases equation (16) is to be replaced by

$$\frac{\partial c}{\partial t} = \frac{\partial c}{\partial x} \left( D \frac{\partial c}{\partial x} \right) \cdot \cdot \cdot \cdot \cdot \cdot \quad (19)$$

L. Boltzmann<sup>28</sup> has suggested that where  $c$  is a function only of  $y' = x/\sqrt{t}$ , this equation may be integrated to give the relation

$$D = -\frac{1}{2} \cdot \frac{dy'}{dc} \int_0^c y' \cdot dc \cdot \cdot \cdot \cdot \cdot \cdot \quad (20)$$

where  $D$  is a differential diffusion coefficient corresponding to a definite concentration  $c$  rather than an integral value for a range of concentrations which would be obtained by the application of procedures based upon equation (16) to concentration-dependent systems. Equation (20) is the basis of the treatment of concentration-dependent systems which will be mentioned again in Part II of this review.

It has been stated that in the experimental determination of diffusion coefficients, the absence of boundary disturbances is essential. Such disturbances cannot, in fact, ever be completely absent, and their occurrence even to a small extent probably sets an upper limit to the size of molecule whose diffusion coefficient can be measured. Coefficients less than  $10^{-7}$  c.g.s. unit have, however, been determined by means of observations extending over several days. Clearly, there is no corresponding lower limit to the size of particles whose diffusion may be investigated, though the boundary disturbances which cannot be avoided on making the boundary may be more troublesome here in preventing the observation of the earlier stages of diffusion.

It is readily shown that, at low concentrations, the diffusion coefficient is related to the frictional constant by the simple equation :

$$D = kT/f = RT/F \cdot \cdot \cdot \cdot \cdot \cdot \quad (21)$$

where  $F = Nf$  is the frictional constant per g.-mol. The determination of diffusion coefficients at low concentration thus provides a ready means of deriving frictional constants.

<sup>28</sup> *Ann. Physik*, 1894, **53**, 959.

For spherical molecules of radius,  $r$ , we have, under certain conditions, by Stokes's law

$$f = 6\pi\eta r \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)$$

where  $\eta$  is the viscosity of the solvent. Expressing  $r$  in terms of the molecular weight  $M$  and the partial specific volume  $V$ , we obtain

$$f = 6\pi\eta\left(\frac{3MV}{4\pi N}\right)^{\frac{1}{3}} \quad . \quad . \quad . \quad . \quad . \quad . \quad (23)$$

Measurement of the diffusion coefficient and therefore of the frictional constant for spherical molecules thus allows a direct calculation of the radius of the molecules and of their molecular weight.

For non-spherical molecules, the position is much more complicated. The frictional constant of an asymmetrical molecule varies with its orientation, three different constants being required, in the case of a generalised ellipsoid, for a complete description of its possible motions.<sup>29</sup> For random orientation, which usually occurs in translational phenomena, the frictional constant has a well-defined intermediate value. Diffusion coefficients at low concentration yield [by equation (21)] such intermediate frictional constants: these cannot, however, be expressed simply in terms of a dimension of the molecules, as in the case of spherical molecules; but if the molecules approximate to some geometrically simple shape, then progress may be made. Thus for ellipsoids of rotation, Perrin<sup>29</sup> has made use of the frictional ratio  $f/f_0$ , where  $f_0$  is the hypothetical frictional constant of an unsolvated spherical molecule identical in molecular weight and volume with the unknown. Such a ratio may be expressed in terms of the ratio of the lengths of the two axes of the ellipsoid, whether it be oblate or prolate. On the basis of Perrin's equations, curves of the frictional ratio against axial ratio have been constructed, and if no other factors are involved, then knowledge of the frictional ratio immediately gives the axial ratio of the ellipsoid.

Usually, however, other factors cannot entirely be excluded. For instance, even in the case of idealised ellipsoidal molecules, appreciable solvation usually occurs, leading to an increased molecular weight and volume as well as to an altered frictional ratio. J. L. Oncley<sup>30</sup> has attempted to allow for such solvation effects by writing the frictional ratio as the product of two factors:

$$f/f_0 = (f/f_e)(f_e/f_0) \quad . \quad . \quad . \quad . \quad . \quad . \quad (24)$$

the first denoting the influence of solvation, and the second that of asymmetry. Considering solvation as merely increasing the effective radius of a spherical molecule, in conformity with Stokes's law, we have

$$f/f_e = (1 + w/V\rho) \quad . \quad . \quad . \quad . \quad . \quad . \quad (25)$$

where  $w$  is the weight of solvent strongly bound by 1 g. of solute of partial specific volume  $V$ . The second factor is given by Perrin's equations. In the

<sup>29</sup> F. Perrin, *J. Phys. Radium*, 1936, **7**, 1.

<sup>30</sup> *Ann. N.Y. Acad. Sci.*, 1941, **41**, 121; see also in "Proteins, Amino Acids and Peptides", by E. J. Cohn and J. T. Edsall, Reinhold, 1943, p. 424.

case of proteins in aqueous solutions, Oncley calculated the curves in Fig. 2 in which the effects of both hydration and asymmetry on the frictional ratio are clearly evident.

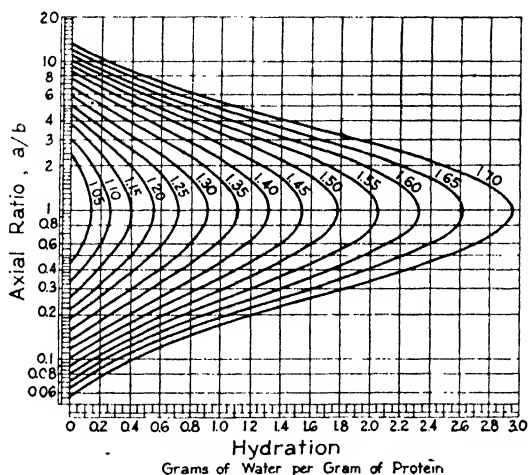


FIG. 2.

*Effects of hydration and axial ratio on the frictional ratios of proteins.*

*(Contour lines denote  $f/f_0$  values.)*

*(Reproduced by permission from Annals of the New York Academy of Sciences, 1941, 41, 121.)*

If the frictional ratio and one of the other variables are known, then the third quantity may be determined. Usually, as indicated later, a reasonable value of the hydration is assumed, and the axial ratio of the assumed ellipsoidal molecule may therefore be calculated.

Whether this procedure of deriving molecular asymmetry, even with ideal experimental data, is quantitatively reliable is not yet known, owing to the absence of alternative sources of information. The method of allowing for solvation is probably not without objections, but it would seem, at the moment, that in view of our lack of precise knowledge of hydration and of experimental error in frictional ratios, the use of more complex methods is not justified.

If the molecules do not approximate to ellipsoids but are more complicated, it is usual to speak of hydrodynamically equivalent ellipsoids: however, the dimensions so calculated may bear little resemblance to the actual dimensions of the solute molecule. In any case many polymers (*e.g.*, the flexible linear chain-like polymers) have no uniquely defined configuration, and any calculated dimensions have only a statistical significance.

In making use of measured frictional constants, the molecular weight must be assumed in obtaining  $f_0$ . Some methods have already been mentioned, but sedimentation-velocity measurements are most frequently used in association with those of diffusion.

*Sedimentation velocity.* This method,<sup>14</sup> being concerned with the *rate*

at which polymer molecules or colloidal particles move under the influence of a high centrifugal field, possesses no thermodynamic basis, and is not therefore to be confused with the method of sedimentation equilibrium. It is clearly an extension of the well-known industrial method of investigating particle size by the rate of settling under gravity of coarse colloidal suspensions.

The colloidal solution is contained in a sector-shaped cell, fixed radially in the rotor, which is spun at speeds giving centrifugal fields in the cell of up to 500,000 g. Whereas in the sedimentation-equilibrium method the centrifugal field is chosen to give a balance between sedimentation and diffusion in which the solute concentration varies over the whole cell, the high fields in the velocity method give a sedimentation rate considerably in excess of the diffusion rate throughout the cell. As a first approximation, therefore, diffusion may be neglected, and in sedimentation the whole of the solute molecules move with respect to the solvent in the direction of the centrifugal field, giving rise to a moving boundary between solvent and solution at which an abrupt change in concentration or concentration gradient occurs. Optical methods are used to make observations of the concentration or its gradient throughout the cell at suitable times during sedimentation, from which is derived the sedimentation constant (or rate of sedimentation under unit centrifugal field), given by

$$s = (dx/dt)/w^2x \quad . \quad . \quad . \quad . \quad . \quad (26)$$

where  $x$  is the distance of the moving boundary from the axis of rotation at time  $t$ .

Diffusion, which tends to smooth out concentration differences, causes spreading of the moving boundary, but, providing this is not excessive, it does not prevent the observation of sedimentation constants. In the case of monodisperse polymers (in which all solute molecules for a given field move with the same velocity), the spreading observed may, in favourable cases, be used to calculate approximate diffusion coefficients. However, for systems which are not monodisperse, boundary spreading also arises as a result of the differing velocities of sedimentation of the different molecular species, and clearly diffusion coefficients cannot so be obtained.

Other boundary effects, even in the case of monodisperse solutes, either prevent or seriously hinder the determination of diffusion coefficients. Especially must we mention the boundary sharpening or blurring which arises from the existence of concentration-sensitive sedimentation constants.<sup>14</sup>

If the different molecular species in a paucidisperse system sediment at sufficiently different rates, then the originally single boundary, which spreads rapidly at first, may split into several subsidiary peaks whose sedimentation constants may then be observed. This possibility of measuring sedimentation constants in polymer mixtures, and the analysis of such mixtures in terms of the differently sedimenting components, constitutes one of the important advantages of the sedimentation-velocity over the sedimentation-equilibrium method, which, as was indicated, provides only meagre information on polydisperse systems.

If the sedimentation constant is to be representative of the individual solute molecule only, then it must clearly be independent of concentration effects, or charge effects resulting from the presence of electrolyte. The former are removed by measuring sedimentation constants at low concentration and extrapolating to zero concentration, and the latter by the addition of sufficient electrolyte. The corrected sedimentation constant should then be dependent only upon (a) the size and shape of the sedimenting molecule and (b) the nature of the solvent medium. Such sedimentation constants only can be simply related to the properties of the individual molecule.

It may readily be shown,<sup>31</sup> by equating the frictional resistance suffered by a solute molecule moving at its terminal velocity of sedimentation with the centrifugal force acting on it, that the molecular weight and sedimentation constant are related by the expression

$$M = Fs/(1 - V\rho) \quad . \quad . \quad . \quad (27)$$

If  $F$  be known in addition to  $s$ , then the molecular weight may be calculated. The most frequent source of  $F$  is diffusion measurements, and by substituting equation (21) we obtain the well-known equation

$$M = RTs/D(1 - V\rho) \quad . \quad . \quad . \quad (28)$$

It is clear that the molecular weight of a polymer may be obtained if, in addition to the routine determination of  $V$  and  $\rho$ , the sedimentation constant and diffusion coefficient at zero concentration are determined. No assumption as to the shape of the sedimenting molecule is contained in equation (28); it is assumed, however, that the frictional constants in diffusion and sedimentation are identical, which is reasonable in view of the random orientation usually common to both processes.

It should be noted that the molecular weight so calculated refers not to the solvated but to the unsolvated molecule, in spite of the fact that the sedimenting species is undoubtedly solvated.<sup>32</sup> This result arises from the fact that the partial specific volume, as normally determined, refers to the unsolvated molecule. Nor does equation (28) yield a well-defined mean molecular weight in the case of a polydisperse polymer,<sup>10</sup> and the use of well-fractionated materials is therefore strongly recommended.

Having thus determined the molecular weight, the hypothetical frictional constant,  $f_0$ , for the unsolvated spherical molecule may be calculated from equation (23), and the frictional ratio obtained. Typical values will be quoted in Part II, with the resulting estimates of particle shape.

With centrifugal fields limited to *ca.* 500,000 *g.* there is a definite lower limit of particle size which can be investigated by the sedimentation velocity method. For proteins, the lower limit in terms of molecular weight is *ca.* 10,000. There is no upper limit for the method, providing the frictional constants in sedimentation and diffusion can be considered identical.

**Electrophoresis.**—Although primarily used to investigate the electrical

<sup>31</sup> Svedberg and Pedersen, *op. cit.*, p. 5.

<sup>32</sup> E. O. Kraemer, in "The Ultracentrifuge" (ref. 14), p. 57.

charge rather than the size or shape of polymer molecules in solution, useful information on the shape of certain protein molecules has been obtained recently from electrophoretic measurements.<sup>33, 34</sup> Only the principles of the method of calculation can be mentioned here.

Experimental measurements yield electrophoretic mobility directly usually as a function of pH. If the molecular weight, as determined by some other method, be assumed, and a certain degree of asymmetry be postulated, it is then possible to convert the mobility-pH curve into a curve of net charge against pH.

A similar curve can also be determined directly for proteins by a determination of acid and base binding capacity as a function of pH, since under certain conditions the absorption of ions other than H<sup>+</sup> and OH<sup>-</sup> may be neglected, the number of hydrogen or hydroxyl ions bound per molecule then giving the net positive or negative charge.<sup>35</sup>

The curve thus obtained from acid and base titrations may be compared with that derived from electrophoretic measurements, different degrees of asymmetry being assumed, and the actual molecular asymmetry being accepted as that giving the best agreement with the titration curve.

Clearly, the method is limited to charged polymer molecules, and as yet has been applied only in a qualitative manner.

*Rotational diffusion.* Observations of the rotational motion of polymer molecules occurring in opposition to some orientating force may be used to investigate molecular size and dimensions. The orientating force is usually either (a) a velocity gradient in a liquid,<sup>36</sup> or (b) an alternating electrical field,<sup>37</sup> the former method being more suitable for the larger and more asymmetrical particles (e.g., tobacco mosaic virus), whilst the latter, with the possibility of fields of very high frequency (up to 10<sup>10</sup>–10<sup>11</sup> cycles per sec.), has been used for the almost spherical molecules.

Consider a system of non-spherical molecules whose orientation is described by means of the angle ( $\phi$ ) made by some definite axis in the molecule with a fixed direction in space. If  $\Delta n$  molecules per unit volume have this axis within the directions  $\phi$  and  $\phi + \Delta\phi$ , then it is possible to speak of a distribution function or "angular concentration"  $\lambda(\phi)$ , defined as  $\frac{\Delta n}{\Delta\phi}$ .

$$\frac{\Delta n}{\Delta\phi}$$

A coefficient of rotational diffusion ( $\Theta$ ) is then defined [cf. equation (15)] by

$$dn = \Theta(d\lambda/d\phi)dt \quad . \quad . \quad . \quad (29)$$

where  $dn$  is the number of molecules whose axes move through the position

<sup>33</sup> L. S. Moyer and M. H. Gorin, *J. Gen. Physiol.*, 1941–1942, **25**, 785.

<sup>34</sup> H. A. Abramson, L. S. Moyer, and M. H. Gorin, "Electrophoresis of Proteins", Reinhold, 1942.

<sup>35</sup> R. K. Cannan, *Chem. Reviews*, 1942, **30**, 395.

<sup>36</sup> See review by J. T. Edsall, "Advances in Colloid Chemistry", Vol. I, Interscience Publishers, 1942, p. 269; also in "Proteins, Amino Acids and Peptides", 1943, p. 506.

<sup>37</sup> J. L. Oncley, *Chem. Reviews*, 1942, **30**, 433.

$\phi$  to higher values in time  $dt$ . The rotational diffusion coefficient is also contained in the differential equation [analogous to equation (16)]

$$d\lambda/dt = \Theta(\partial^2\lambda/\partial\phi^2) \quad . \quad . \quad . \quad . \quad . \quad (30)$$

with whose solutions we are concerned experimentally.

As in translational diffusion,  $\Theta$  may be related to a rotational frictional constant  $\xi$ , by an equation

$$\Theta = kT/\xi \quad . \quad . \quad . \quad . \quad . \quad . \quad (31)$$

$\xi$  being itself defined by the general equation (13). Determination of  $\Theta$  thus gives  $\xi$  directly, and again as in the translational case this, for spherical molecules, gives the molecular radius by the rotational form of Stokes's law :

$$\xi = 8\pi\eta r^3 \quad . \quad . \quad . \quad . \quad . \quad . \quad (32)$$

F. Perrin<sup>38</sup> has also expressed the frictional ratio,  $\xi/\xi_0 (= \Theta_0/\Theta)$ , in terms of the appropriate ellipsoidal model. If the molecular weight is known in addition to  $\xi$ ,  $\xi_0$  may be calculated, and from it the frictional ratio, which may then be used to obtain the axial ratio of the ellipsoidal molecule. Alternatively,  $\xi_0$  may be expressed in terms of the ellipsoid axes and an equation of the type

$$\Theta_b = \frac{3kT}{16\pi\eta a^3} \cdot [2 \ln 2a/b - 1] \quad . \quad . \quad . \quad . \quad . \quad (33)$$

may be obtained which in this case refers to the rotation of the axis of revolution (of length  $2a$ ) about the equatorial axis (of length  $2b$ ) for an elongated ellipsoid of revolution. Since  $\ln 2a/b$  varies slowly, an approximate value of  $a/b$  may first be substituted to give a preliminary value of  $a$ , which may further be refined by inserting more accurate values of  $a/b$ .

Other expressions are available for other modes of rotation and for oblate ellipsoids of rotation. As in the translational case, the significance of the dimensions calculated depends upon the extent to which the actual molecule corresponds to the assumed model. One difference, however, is to be noted. The results obtained here refer to the solvated molecule or, in other words, to the actual kinetic unit as it occurs in solution. It should be recalled that molecular weights obtained from sedimentation and diffusion refer to the unsolvated molecule because of the use of the unsolvated partial specific volume : the frictional constant, even in this case, however, refers to the solvated molecule. For polydisperse materials, the measured rotational diffusion coefficient may vary with the velocity gradient, and as in the previously discussed dynamic methods, the use of fractionated materials has great advantages.

*Orientation in a velocity gradient.* A velocity gradient is usually provided in some form of concentric cylinder apparatus,<sup>39, 40</sup> in which one of the cylinders is rotated smoothly with respect to the other, from which it is separated by a narrow annular space containing the polymer solution. The magnitude

<sup>38</sup> *J. Phys. Radium*, 1935, **5**, 497.

<sup>39</sup> J. R. Robinson, *Proc. Roy. Soc.*, 1939, **A**, **170**, 519.

<sup>40</sup> J. T. Edsall *et al.*, *Rev. Sci. Instr.*, 1944, **15**, 243.

of the velocity gradient ( $G$ ) is readily calculated from the rate of rotation and from the dimensions of the apparatus.

The velocity gradient causes orientation by swinging the asymmetrical particles into the direction of the stream-lines, rotational diffusion opposing this process. Orientation is to be regarded as a dynamic process in which the direction of maximum orientation is merely that direction through which the particles move most slowly, though, at equilibrium, the rate at which particles enter this direction is equal to the rate at which they leave it. The degree of orientation depends upon the relative magnitudes of the gradient and the rotational diffusion coefficient of the particles, and is usually followed by means of the streaming birefringence caused by the orientation of anisotropic particles. Observations on the cross of isocline yield the angle ( $\chi$ ) made by the direction of maximum orientation with the streamlines in the flowing liquid. This angle, which varies from  $0^\circ$  to  $45^\circ$  as  $G/\Theta (= \alpha)$  changes from infinitely large to infinitely small values, may be used directly to calculate the rotational diffusion coefficient ( $= G/\alpha$ ) by means of the equations of P. Boeder,<sup>41</sup> or A. Peterlin and H. A. Stuart,<sup>42</sup> which have been obtained as special solutions of the general differential equation (30), and which at low values of  $\alpha$  reduce to

$$\chi = \pi/4 - \alpha/12 \quad . \quad . \quad . \quad . \quad . \quad (34)$$

The value of the rotational diffusion coefficient may then be used to derive particle dimensions by equation (33) or similar equations, providing it is justifiable to treat the particle in terms of a rotational ellipsoid or some other geometrically simple model. Results so derived will be discussed later.

The practical difficulties limiting the velocity gradients attainable to about *ca.* 50,000 c.g.s. units clearly set an upper limit of *ca.* 20,000 sec.<sup>-1</sup> to the diffusion coefficients which can be obtained by this method. For greater diffusion coefficients, appreciable orientation cannot thus be obtained; the method employing an alternating electrical field is, however, suitable in such cases.

*Orientation in an alternating electrical field.* At low frequencies of an alternating electrical field (*e.g.*,  $10^2$ — $10^4$  cycles per sec.), a dipolar colloidal molecule in solution can follow the reversals of the field and can therefore make its maximum contribution to the dielectric constant of the solution. At very high frequencies (*e.g.*, *ca.*  $10^8$ — $10^9$  cycles per sec.), the period of the field is much smaller than the time required for the orientation of such dipoles, which do not therefore contribute to the measured dielectric constant; further, since they displace smaller solvent molecules which would achieve orientation within the period of the field, the presence of the colloid may result in a lowered dielectric constant at high frequency. At intermediate frequencies, only a fraction of the dipoles contribute to the dielectric constant of the solution, the fraction decreasing continuously from the value unity at low frequencies to zero at high frequencies.

In treating dielectric dispersion quantitatively, it is usual to make use of

<sup>41</sup> *Z. Physik*, 1932, **75**, 259.

<sup>42</sup> *Ibid.*, 1939, **112**, 1, 129.



the relaxation time,  $\tau$ , which gives a measure of the time required, in a system of initially orientated molecules, for the mean value of  $\cos \phi$  ( $\phi$  being the angle made by the axis of orientation with a direction of orientation) to fall to  $1/e$ .  $\tau$  is clearly related to the rotational frictional constant and diffusion coefficient, and for the relaxation time  $\tau_a$ , characterizing the rotation of the  $a$  axis about the  $b$  and  $c$  axes of a generalized ellipsoid, this relation is quantitatively expressed by

$$\tau_a = 1/(\Theta_b + \Theta_c) \quad . \quad . \quad . \quad (35)$$

which in the special case of a rotational ellipsoid ( $b = c$ ) becomes

$$\tau_a = 1/2\Theta_b \quad . \quad . \quad . \quad (36)$$

Where orientation involves only one relaxation time, the variation in dielectric constant with frequency is given<sup>43,44</sup> by

$$D = D_\infty + (D_0 - D_\infty)/(1 + \nu^2/\nu_c^2) \quad . \quad . \quad . \quad (37)$$

$D_0$  and  $D_\infty$  being the (constant) dielectric constants at very low and very high frequency,  $\nu$  being the frequency of the applied field, and  $\nu_c$  a critical frequency defined by  $\nu_c = 1/2\pi\tau$ , for which  $D = \frac{1}{2}(D_0 + D_\infty)$ . Clearly, therefore, from experimentally observed curves of dielectric constant against frequency, the critical frequency may be obtained, and from it the rotational diffusion coefficient.

Where orientation involves more than one relaxation time, an equation of the type

$$D = D_\infty + \frac{\Delta D_a}{1 + \nu^2/\nu_a^2} + \frac{\Delta D_b}{1 + \nu^2/\nu_b^2} + \dots \quad . \quad . \quad . \quad (38)$$

is required, and although this is more difficult to use than equation (37), values of  $\nu_a$  and  $\nu_b$  may be obtained. Usually the experimental curves are compared with curves calculated on the basis of an assumed molecular weight (usually derived from other sources), axial ratio and partition of  $\Delta D_i (= D_0 - D_\infty)$  into  $\Delta D_a$  and  $\Delta D_b$ . The axial ratio value giving the closest correspondence is then accepted. Alternative methods of calculation may, however, be used.

*Viscosity.*—Although much used and quoted as a suitable method for the determination of molecular weight and dimensions, viscosity can yet be regarded only as a secondary method, whose successful application is dependent upon careful calibration against primary methods of the type already discussed.

In the light of much recent work,<sup>45-48</sup> it is now generally accepted that the well-known Staudinger law, which may be written in the form

$$\lim_{c \rightarrow 0} \eta_{sp}/c = KM \quad . \quad . \quad . \quad (39)$$

<sup>43</sup> P. Debye, "Polar Molecules", Reinhold, 1929.

<sup>44</sup> J. Wyman, *Chem. Reviews*, 1936, **19**, 213.

<sup>45</sup> J. M. Burgers, Second Report on Viscosity and Plasticity, Acad. Sci., Amsterdam.

<sup>46</sup> M. L. Huggins in "Cellulose and Cellulose Derivatives", edited by E. Ott, Interscience Publishers, 1943, p. 943.

<sup>47</sup> R. Fordyce and H. Hibbert, *J. Amer. Chem. Soc.*, 1939, **61**, 1910.

<sup>48</sup> P. J. Flory, *ibid.*, 1943, **65**, 372.

$\eta_{sp.}$  being the specific viscosity and  $K$  a constant for the system, gives only a qualitative indication of molecular weight. A modified form of this law, for which considerable experimental evidence has been provided, *viz.*,

$$\lim_{c \rightarrow 0} \eta_{sp.}/c = KM^\alpha \quad . \quad . \quad . \quad (40)$$

has, however, been suggested,<sup>48,49</sup> in which the additional constant  $\alpha$ , varying between 0.5 and 1.5, requires independent evaluation for every system. With carefully determined values of  $K$  and  $\alpha$ , this equation probably represents the most valuable method of obtaining molecular weight from viscosity data.

Of the many equations relating viscosity and particle dimensions,<sup>50</sup> we can mention only the recent one of R. Simha,<sup>51</sup> developed for a suspension of rigid ellipsoidal particles in overwhelming Brownian motion, a condition which usually (though not always) holds for polymer solutions. Writing  $a/b = J$ , and  $\phi$  = volume concentration, we obtain

$$\eta_{sp.}/\phi = \frac{J^2}{15 (\ln 2J - 3/2)} + \frac{J^2}{5 (\ln 2J - 1/2)} + \frac{14}{15} \quad . \quad . \quad (41)$$

It is valid only at low concentrations, and as with other methods of obtaining information on particle dimensions, it can only have physical significance if the real particle approximates closely to the postulated model. As in the determination of axial ratios from frictional ratios, uncertainty in the degree of solvation gives corresponding uncertainty in calculated values of the axial ratio.

(3) *Miscellaneous Methods.—Optical.* Although it has long been known that the optical properties of colloidal solutions may give qualitative information on the nature of the colloidal particles, it is only recently that the investigation of the nature and intensity of the scattered light has been used to provide quantitative information on the molecular weight and dimensions of dissolved polymers.

P. Putzeys and J. Brosteaux<sup>52</sup> measured the intensity of light scattered at 90° to the incident direction from solutions of several globular proteins and, with amandin as a standardising material, used the well-known Rayleigh equation, suitably extended to correct for depolarisation :

$$\frac{I}{I_0} = \frac{9\pi^2}{2\lambda^4 N} \left[ \frac{n_2^2 - n_1^2}{n_2^2 + 2n_1^2} \right]^2 \frac{6}{6 - 7\rho_u} V^2 c M \quad . \quad . \quad (42)$$

to evaluate other molecular weights;  $I_0$  is the intensity of the incident light of wave-length  $\lambda$ ,  $I$  that of the light scattered from unit volume of solution,  $N$  is the Avogadro number,  $n_1$  and  $n_2$  are the refractive indices of solvent and protein respectively, and  $\rho_u$  the depolarisation ratio. Values so obtained are

<sup>48</sup> T. Alfrey, A. Bartovics, and H. Mark, *J. Amer. Chem. Soc.*, 1939, **61**, 2319.

<sup>49</sup> F. Eirich, *Ann. Rep. Prog. Physics*, 1940, **7**, 329; see also H. Mark, "High Polymers", Vol. II, Interscience Publishers, 1940, p. 258.

<sup>50</sup> *J. Physical Chem.*, 1940, **44**, 25.

<sup>51</sup> *Trans. Faraday Soc.*, 1935, **31**, 314; *Med. Koninkl. Vlaasche Acad. Belg.*, 1941, III, No. 1.

shown in Part II to be in reasonable agreement with those established by other methods.

The alternative method of using light-scattering measurements to investigate the thermodynamic properties of polymer solutions has already been mentioned (p. 33) and will not be further discussed here. A comparison of the two methods is clearly required.

When a dimension of the polymer molecule is comparable with the wavelength of the light employed, the distribution of scattered intensities is found to be no longer symmetrical about the  $90^\circ$  position.<sup>53</sup> Measurements of the scattered intensities ( $I_1$  and  $I_2$ ) at two angles  $\theta_1$  and  $\theta_2$ , symmetrically placed about the  $90^\circ$  position, yield the disymmetry coefficient  $q$  defined by

$$q = I_1/I_2 - 1 \quad . \quad . \quad . \quad . \quad . \quad . \quad (43)$$

If a definite molecular model be postulated, it is possible to calculate the variation of  $q$  with the molecular dimensions of the model and therefore to determine model dimensions from observed  $q$  values. A preliminary test of this method, performed on tobacco mosaic virus protein, whose dimensions have been accurately determined by the electron microscope, has given favourable indications of the method. Further results so obtained by P. Doty, W. A. Affens, and B. H. Zimm<sup>54</sup> for polystyrene in solution will be mentioned in Part II.

A further method of investigating the form of polymer molecules in solution utilizes measurements of the depolarisation ratios

$$\rho_u = \frac{H_u}{V_u}, \quad \rho_v = \frac{H_v}{V_v}, \quad \text{and} \quad \rho_h = \frac{H_h}{V_h} \quad . \quad . \quad . \quad . \quad (44)$$

where  $H$  and  $V$  respectively denote the intensities of the horizontal and vertical components of the scattered light, and the subscripts  $u$ ,  $v$ , and  $h$  refer to unpolarized, vertically polarized, and horizontally polarized incident light.<sup>55</sup> Although not yet suitable for absolute measurements of molecular dimensions, the method is convenient for detecting changes in molecular form.

*X-Ray and electron diffraction.* In the case of certain proteins which form single crystals, X-ray evaluation of the dimensions and shape of the unit cell may give valuable information on molecular weight and shape in the crystal,<sup>56</sup> as well as indicate the order of magnitude of crystal hydration, which has some bearing on the problem of hydration in solution for which, as we have seen, information is urgently required.

Molecular weight is related to the volume of the unit cell ( $v$ ) by the expression :

$$M = N\rho v/n \quad . \quad . \quad . \quad . \quad . \quad . \quad (45)$$

<sup>53</sup> B. H. Zimm, R. S. Stein, and P. Doty, *Polymer Bull.*, 1945, **1**, 90.

<sup>54</sup> *Trans. Faraday Soc.*, 1947, in the press (part of a symposium on "Swelling and Shrinking" in Sept., 1946).

<sup>55</sup> P. Doty and H. S. Kaufman, *J. Physical Chem.*, 1945, **49**, 583.

<sup>56</sup> D. Crowfoot, *Chem. Reviews*, 1941, **28**, 215; also M. F. Perutz and J. Boyes-Watson, *Nature*, 1943, **151**, 714.

$n$  being the number of molecules in the unit cell and  $\rho$  the crystal density. Usually equation (45) is used to give a precise molecular weight, after an approximate value, derived by other means, has enabled the determination of  $n$ .

From the shape of the unit cell and the number of molecules contained in it, an estimate of the dimensions of the molecule may be made from packing considerations. Such estimates are usually of a qualitative nature and, being determined for the crystal, may not be applicable directly to the molecule in solution. Similar difficulty is associated also with crystal hydration.

For the less perfect crystals of many linear polymers,<sup>57</sup> a single molecular chain may pass through a large number of unit cells and no indications of molecular weight or shape of the isolated molecule can be obtained from X-ray data; nor do the estimates of crystallite size, as determined by the breadth of X-ray reflections or by the low-angle scattering of X-rays, have any direct bearing on the determination of molecular weight or shape.

The most direct of all methods of deriving molecular dimensions is provided by the electron microscope,<sup>58</sup> in which individual molecules or particles, deposited from dilute solution to minimize aggregation, are photographed under known high magnification. Valuable results have already been obtained, especially for the larger and more asymmetric colloidal particles (*e.g.*, colloidal gold particles and tobacco mosaic virus). For the smaller colloidal molecules whose dimensions are comparable with the resolving power of the electron microscope (*ca.* 20 Å.) and where all possible molecular orientations are encountered, the difficulties are much greater. Nevertheless, recent technical improvements, and especially the use of the atom shadowing technique,<sup>59</sup> give considerable encouragement. In utilizing electron-microscope results, it must, however, be remembered that the process of depositing the unknown material from solution may alter it irreversibly and the dangers of aggregation and electron bombardment should not be under-estimated.

*Analytical methods.* Analytical methods have, in individual cases, provided valuable estimates of molecular weight as well as indications of polymerisation mechanism. In the case of the proteins, trace element (or group) analysis has been particularly useful, as for instance in the case of several haemoglobins for which an accurate estimate of molecular weight was obtained as that weight of protein containing 4 g.-atoms of iron. Similarly useful indications have been obtained from the content of sulphur in haemoglobins, of copper in the haemocyanins, and of trace amino-acids in certain other proteins.<sup>60</sup>

The method of end-group analysis has, as is well known, been employed to investigate the chain length of many polymeric carbohydrates. It was

<sup>57</sup> C. W. Bunn, in "Advances in Colloid Science", Vol. II, Interscience Publishers, 1946, p. 95.

<sup>58</sup> See, *e.g.*, G. D. Preston, *Ann. Reports*, 1944, **41**, 81.

<sup>59</sup> R. C. Williams and R. W. G. Wyckoff, *J. Appl. Physics*, 1944, **15**, 712.

<sup>60</sup> See review by E. J. Cohn, J. L. Hendry, and A. M. Prentiss, *J. Biol. Chem.*, 1925, **63**, 721.

thus shown in the case of cellulose that the endless chain structure did not occur and a lower limit to the degree of polymerisation was obtained.<sup>61</sup> W. H. Carothers and F. J. van Natta<sup>62</sup> similarly investigated poly  $\omega$ -hydroxydecoic acid,  $\text{OH}\cdot([\text{CH}_2]_9\cdot\text{CO}\cdot\text{O})_n\cdot[\text{CH}_2]_9\cdot\text{CO}_2\text{H}$ , by titration with alkali, and the Zerewitinoff procedure, by which OH and SH groups occurring in a polymer are estimated by reaction with a Grignard compound, is of considerable use in investigating the degree and mechanism of polymerisation.<sup>63</sup>

To be successfully employed, all analytical techniques require that the unknown substance can be carefully purified without decomposition, and the end-group method further requires the assumption of a structural model. Clearly, such methods which, in general, yield number average values, have limited but by no means negligible application.

### *Part II. Results.*

Before reviewing results, two difficulties must again be emphasized. The first arises from our meagre knowledge of the extent of solvation, for the study of which adequate experimental methods are not available. In the case of the proteins, a reasonable figure, based upon a considerable number of estimates of crystal hydration,<sup>64</sup> is 0.30 g. of water per g. of dry protein. This figure may, however, need revision. For many other polymers no estimates are available and considerable uncertainty in molecular dimensions is therefore unavoidable.

The second difficulty arises from the complex nature of many polymer molecules, their treatment as geometrically simple shapes being at the best a rather poor approximation. As we have already stressed, the dimensions quoted in the following pages are those of the hydrodynamically equivalent ellipsoid, rod, or other chosen model, and in view of fluctuations in shape of many polymers, they are statistically determined.

It is convenient to consider results under two headings: (a) corpuscular molecules, typified by the corpuscular proteins; and (b) asymmetric molecules, typified by the linear chain-like polymers. In many ways the asymmetric molecules are more complicated in behaviour than those of almost spherical form, and the latter will therefore be first considered.

(a) *Corpuscular Molecules.*—The greater part of the work involving the precise determination of molecular size and shape has concerned the proteins which, with their frequently corpuscular and reproducible molecular form and their monodispersity, are well suited for the application of the techniques just described. The biological rôle of these materials has given added importance to this work.

<sup>61</sup> W. N. Haworth and H. Machemer, *J.*, 1932, 2270.

<sup>62</sup> *J. Amer. Chem. Soc.*, 1933, 55, 4714.

<sup>63</sup> J. L. Bolland, Publication No. 8, (iii), of British Rubber Producers' Research Association.

<sup>64</sup> G. S. Adair, *Ann. Rev. Biochem.*, 1937, 6, 163; see also T. L. McMeekin and R. C. Warner, *J. Amer. Chem. Soc.*, 1942, 64, 2395.

Table III contains the "dry" molecular weights of a selection of proteins as determined by the different techniques.

TABLE III.  
*Molecular weights of corpuscular proteins.*

Protein.	Method.				
	Osmotic pressure. <sup>a</sup>	Sedimentation equilibrium. <sup>b</sup>	Sedimentation and diffusion. <sup>b</sup>	X-Rays. <sup>c</sup>	Light scattering. <sup>d</sup>
Pepsin .....	36,000	39,000	35,500	—	—
$\beta$ -Lactoglobulin .....	—	38,000	41,500	40,000	—
Egg albumin .....	44,000 *	40,500	44,000	—	38,000
Hæmoglobin (horse) ...	67,000	68,000	68,000	66,700	—
Serum albumin (horse)	73,000	68,000	70,000	—	74,000
Serum globulin (horse)	174,000	150,000	167,000	—	—
Excelsin .....	214,000	—	295,000	305,800	280,000
Amandin .....	206,000	330,000	330,000	—	Standard

<sup>a</sup> Cohn and Edsall, *op. cit.*, p. 390.

<sup>b</sup> Svedberg and Pedersen, *op. cit.*, p. 406.

<sup>c</sup> Fankuchen in "Advances in Protein Chemistry", Vol. II, p. 387.

<sup>d</sup> Putzeys and Brosteaux, *loc. cit.*, ref. (52).

\* Mean of the recent osmotic investigations quoted in reference (a).

The considerable measure of agreement shown by the values of Table III, derived by methods differing profoundly in technique and origin, gives considerable support for the methods individually. Although discrepancies undoubtedly occur and further work is certainly required, in which conditions of examination are carefully standardized, the essential soundness of the methods for molecular weight determinations is adequately demonstrated.

From molecular-weight determinations, T. Svedberg and K. O. Pedersen<sup>65</sup> were led to postulate that the molecular weights of proteins were approximate multiples of the unit 17,600. Although many molecular weights (*e.g.*, see Table III) are in agreement with this, there are many exceptions, as has been clearly shown by H. B. Bull's device<sup>66</sup> of plotting a "logarithmic spectrum" of molecular weights and by the statistical analysis of molecular weight data.<sup>67</sup> The hypothesis is therefore under considerable suspicion.

A modified form of this idea was, however, contained in the hypothesis of M. Bergmann and C. Niemann,<sup>68</sup> by which the total number of amino-acids in a protein molecule (rather than its molecular weight) is expressible in the form  $2^m \times 3^n$ , where  $m$  and  $n$  are integers greater than zero. Clearly, variations in the mean amino-acid residue weight cause variations in molecular weight for molecules containing the same number of amino-acids, so that a defect of the Svedberg multiple law is avoided. However, serious objections

<sup>65</sup> *Op. cit.*, p. 406.

<sup>66</sup> In "Advances in Enzymology", Vol. I, 1941, Interscience Publishers, p. 1.

<sup>67</sup> J. P. Johnston, H. C. Longuet-Higgins, and A. G. Ogston, *Trans. Faraday Soc.*, 1945, **41**, 588.

<sup>68</sup> *J. Biol. Chem.*, 1936, **115**, 77; 1938, **122**, 577.

are also raised on statistical<sup>69</sup> and on analytical grounds,<sup>70</sup> and the Bergmann-Niemann hypothesis, like its predecessor, is regarded with some doubt.

A considerable body of information on the shape of the corpuscular protein molecules, derived chiefly from the dynamic methods, is also now available. In addition to the molecular-weight data ( $M_s$ ) and the experimental sedimentation constants and diffusion coefficients from which these were derived, Table IV contains the frictional ratios obtained similarly, and axial ratios derived therefrom on the basis of zero and 30% hydration, assuming an elongated ellipsoid of rotation. For comparison, axial ratios derived, by using the same assumptions, from viscosity data by means of Oncley's curves<sup>30</sup> based on Simha's equation (p. 49) are included.

The choice of a prolate (rather than oblate) ellipsoid of rotation is somewhat arbitrary; similar agreement is, however, observed if the oblate ellipsoid is assumed, and on the basis of these results a choice between the two forms is impossible (see, however, D. G. Dervichian,<sup>71</sup> J. F. Foster, and J. T. Edsall<sup>72</sup>).

TABLE IV.

*The molecular weight and axial ratio of certain proteins.*

Protein.	$\times 10^{13}$ .	$\times 10^{17}$ .	$M_s$ .	$f/f_0$ .	Axial ratios.			
					From $f/f_0$ :		From viscosity:	
					0% hydr- ation.	30% hydr- ation.	0% hydr- ation.	30% hydr- ation.
	(a.)	(a.)	(a.)	(a.)	(b.)	(b.)	(c.)	(c.)
Pepsin .....	3.3	9.0	35,500	1.08	2.8	—	4.5	3.2
Lactoglobulin .....	3.12	7.3	41,500	1.26	5.1	3.2	5.1	3.6
Egg albumin .....	3.55	7.8	44,000	1.16	3.8	1.9	4.8	3.4
Hæmoglobin (man) .....	4.48	6.9	63,000	1.16	3.8	1.9	4.6	3.2
Serum albumin (horse) ...	4.46	6.1	70,000	1.27	5.3	3.4	5.5	4.0
Serum globulin (horse) ...	7.1	4.05	167,000	1.44	8.3	5.7	7.5	5.7
Excelsin .....	13.3	4.26	295,000	1.13	3.4	1.5	—	—
Amandin .....	12.5	3.62	330,000	1.28	5.5	3.5	6.0	4.5
Thyroglobulin (pig) .....	19.2	2.65	630,000	1.43	8.1	5.7	7.7	6.0

(a) Svedberg and Pedersen, *op. cit.*, p. 406.

(b) Calculated from data in col. 4 by Oncley's curves (Fig. 2).

(c) Calculated from data of A. Polson (*Kolloid-Z.*, 1939, **88**, 51) by Oncley's curves (ref. 30).

In view of our lack of precise knowledge regarding the contribution of hydration to either the frictional constant or the intrinsic viscosity ( $[\eta]_{sp}/c = 0$ ), the qualitative agreement in the axial ratios is encouraging. More quantitative agreement awaits information on hydration rather than further increase in experimental accuracy.

Oncley<sup>30</sup> has attempted to define the limits within which the axial ratio of certain well-known proteins must lie by assuming certain values of the

<sup>69</sup> See, e.g., A. Neuberger, *Proc. Roy. Soc.*, 1939, **A**, **170**, 64.

<sup>70</sup> A. C. Chibnall, *Proc. Roy. Soc.*, 1942, **B**, **131**, 136.

<sup>71</sup> *J. Chem. Physics*, 1943, **11**, 236.

experimental error and all possible values of hydration from zero upwards. He has thus obtained the curves of Fig. 3, in which the different shaded areas represent the possible values of the axial ratio as determined by the different methods. Clearly, those areas common to the greatest number of methods denote the most probable values of the axial ratio, and on the basis of the curves the prolate form of the ellipsoid would appear the better representation of the protein molecules.

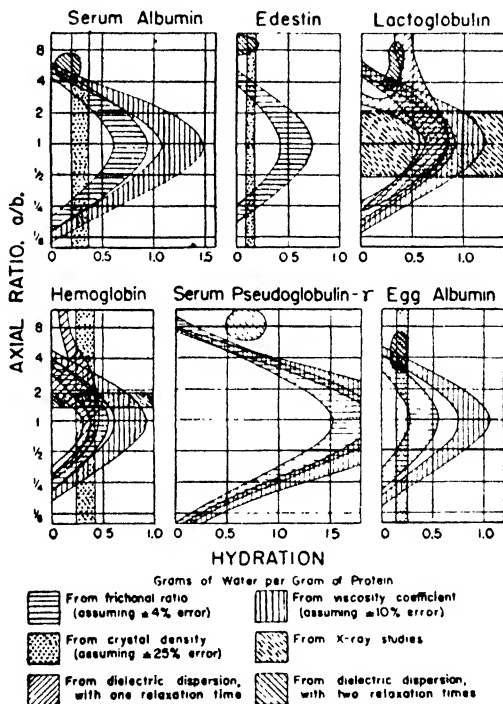


FIG. 3.

*The asymmetry and hydration of certain proteins.*

(Reproduced by permission from *Annals of the New York Academy of Sciences*, 1911, 41, 121.)

The most symmetrical protein molecules, with their comparatively large rotational diffusion coefficients, are not readily studied by streaming birefringence, but the more asymmetric maize protein, zein, has been carefully investigated by this method. Foster and Edsall<sup>72</sup> thus concluded that the zein molecule approximates to a prolate ellipsoid of revolution of length 300–400 Å. and axial ratio *ca.* 20, an oblate ellipsoid being impossible to reconcile with their experimental results. For certain other of the more asymmetric protein molecules (*e.g.*, *Helix hæmocyannin*), lengths of the molecule deduced from flow-birefringence measurements are in reasonable agreement

<sup>72</sup> *J. Amer. Chem. Soc.*, 1945, 67, 617.



with those derived from frictional ratios, prolate ellipsoids of revolution being assumed.<sup>73</sup>

Summarising the position, it may be said that agreement between the different methods as to the shape of the corpuscular protein molecules is as good as can be expected in absence of precise information on hydration, giving further support (see p. 53) for the theoretical basis of the methods and for the treatment of the molecules in terms of geometrically simple models. From the agreement between the methods involving translational and rotational motion of the particles, it appears that the shape of the corpuscular protein molecule is uninfluenced by the presence of velocity gradients; further, in orientation in an electrical field, rotation of the whole rigid molecule, rather than of parts, occurs. This result is to be contrasted with the segmental motion of many linear polymer molecules.

One further point arising from the almost spherical and compact molecular form of the corpuscular proteins should be mentioned. Possessing a relatively small effective volume in solution, such solute molecules interact rarely except at the higher protein concentrations. The variation in sedimentation constant or diffusion coefficient with concentration is therefore not great in dilute solution (1–2%) and extrapolation to zero concentration is not therefore difficult. The variation is, however, by no means negligible,<sup>74</sup> and must be allowed for in accurate work. A variation of much greater magnitude is, however, encountered with the asymmetric molecules, now to be considered.

(b) *Asymmetric Molecules*.—In this group is to be considered the greater part of both the naturally occurring and the synthetic high polymers, many of which possess molecules of the linear chain-like type, with varying degrees of flexibility (*e.g.*, cellulose, unvulcanized rubber, vinyl polymers) or aggregates of such chains (*e.g.*, starch, glycogen). In some cases cross linkages between reactive points on the same or different chains may occur, while in others the kinking of the main chain is unimpeded by such bonds.

Such polymers are usually polydisperse over wide limits, and although the mean molecular weight and range are of some interest for particular samples of material, it is clear that these molecular weights do not have the same general significance as in the case of the monodisperse corpuscular proteins. Measurement of such molecular weights by several methods, usually for carefully fractionated materials, is, however, of importance in testing the basic assumptions of the methods for molecules of complex form. In addition to polydispersity, we have the further complication of non-specific molecular configuration, variations in the configuration of the same molecule under different conditions and at different times being possible as well as between molecules of different molecular weight. The treatment of such molecules in solution as geometrically simple shapes is, as already mentioned, more open to question than in the case of monodisperse corpuscular proteins, and therefore requires more careful testing.

<sup>73</sup> E. T. Cohn and J. T. Edsall, *op. cit.*, p. 541.

<sup>74</sup> P. Johnson, unpublished experiments.

Amongst osmotic investigations of high polymers, the thorough work of Flory<sup>48</sup> on polyisobutylene fractions in cyclohexane and benzene is typical of many. In agreement with the predictions of theory [equation (3)], different polymer fractions give parallel plots of  $\pi/c-c$ ; each plot is not, however, accurately linear, but appears to possess a small curvature which has been confirmed by some workers for other systems (*e.g.*, A. Dobry;<sup>75</sup> G. Gee and L. R. G. Treloar<sup>76</sup>), but questioned by others.<sup>10, 49</sup> Until such uncertainty concerning the exact form of the  $\pi/c-c$  plots disappears, it is clear that in deriving  $\lim_{c \rightarrow 0} \pi/c$ , the range of extrapolation should be minimized by the

use of measurements at the lowest possible solute concentration. Some comparison of molecular weights determined osmotically and otherwise has been made; *e.g.*, Fuoss and Mead<sup>8</sup> obtained for a polyvinyl chloride fraction the molecular weights 100,000 and 102,000 by osmotic and by sedimentation-diffusion measurements, respectively. Clearly, however, extensive comparisons of this type are required, which as yet have largely been held up by the difficulties of working at sufficiently low concentration in sedimentation and diffusion.

In view of the deviations from ideal behaviour to be expected in the sedimentation equilibrium of asymmetric polymers, the analysis of results for substances other than monodisperse or well fractionated samples is of considerable difficulty. For instance, Table V, due to Gralen,<sup>77</sup> gives experimental results for the sedimentation equilibrium of unfractionated cellulose nitrate (from unbleached American linters) in amyl acetate, and molecular weights calculated from equations (8) and (11).

TABLE V.

*Sedimentation equilibrium of cellulose nitrate in amyl acetate.*

Solute concn. = 0.102 g./100 c.c.; speed = 2400 r.p.m.;  $k = 4.2$ ;  $k_1 = 4.2$ .

x.	Z.	$10^6(dn/dx)$ .	c.	M :	
				Equ. (18).	Equ. (11).
4.86	28	16.2	0.0638	426,000	708,000
4.91	28	16.2	0.0734	362,000	642,000
4.96	28	16.2	0.0829	330,000	615,000
5.01	28	16.2	0.0925	291,000	578,000
5.06	28.5	16.5	0.1021	273,000	575,000
5.11	30	17.4	0.1121	234,000	560,000
5.16	32.5	18.9	0.1228	289,000	648,000
5.21	36	20.9	0.1345	270,000	653,000
5.25	42	24.4	0.1451	$M_w$ 300,000	618,000

<sup>75</sup> *J. Chim. physique*, 1935, **32**, 46; *Bull. Soc. chim.*, 1935, **2**, 1882.

<sup>76</sup> *Trans. Faraday Soc.*, 1942, **38**, 47.

<sup>77</sup> Ref. (16), p. 79.

The magnitude of the difference between the last two columns should be noted, as well as the effect of non-ideal behaviour in almost obscuring (in col. 5) the rise in molecular weight with higher  $x$  values, which was to be expected from polydispersity. Galen has also shown the existence of similar serious deviations from the ideal equation for molecular weights less than 100,000. In the last column, for which the improved equation (11) was used, the rise in molecular weight with increasing  $x$  is more evident, but although the equation undoubtedly represents an improvement, it is not yet known to what extent it is quantitatively sound. For two cellulose nitrates, Galen obtained the values 618,000 and 780,000 and 454,000 and 430,000, by the sedimentation-equilibrium and the sedimentation-diffusion procedures, respectively. The equation clearly requires further checking, for if sedimentation equilibrium is to be successfully used for molecular-weight determinations of high-polymer asymmetric molecules, this equation or a further improved form is required.

The asymmetry of high polymer molecules in solution introduces considerable complications (connected with those already considered) into their treatment by the dynamic methods, and as in osmotic-pressure and sedimentation-equilibrium determinations, it is necessary to experiment at the lowest possible solute concentrations. Arising from the relatively large effective volume of an asymmetric polymer molecule in solution compared with that of a spherical molecule of identical molecular volume,<sup>78</sup> it is only at extremely low concentrations that any such molecule can move by translation or rotation without interference from like molecules. At normal solute concentrations ( $\approx 1\%$ ) such interference is highly important, and the equations of sedimentation and of translational and rotational diffusion, developed for single molecules moving independently, no longer apply. Fig. 4 shows the effect of concentration on the sedimentation constant of two cellulose nitrate fractions (of molecular weights *ca.* 300,000 and 100,000) in acetone. Similar curves have been obtained for other asymmetric polymers, *e.g.*, polystyrene, tobacco mosaic virus, nucleic acid (R. Signer and H. Gross;<sup>79</sup> M. A. Lauffer;<sup>80</sup> H. G. Tennant and C. F. Vilbrandt<sup>81</sup>), for some of which a linear plot is claimed for the reciprocal of the sedimentation constant against concentration. As shown in Fig. 4, different polymer fractions show greater differences in sedimentation behaviour the lower the solute concentration; on the other hand, in the case of cellulose nitrates, at concentrations greater than 0.5 g./100 c.c., widely differing fractions sediment at identical rates. It would appear that at such concentrations, intermolecular interactions are so strong that the sedimentation is no longer characteristic of individual molecules, but of an irregular three-dimensional network whose sedimentation properties are not sensitive to the precise molecular length of the constituent molecular chains.<sup>78</sup>

<sup>78</sup> H. Campbell and P. Johnson, *Trans. Faraday Soc.*, 1944, **40**, 221.

<sup>79</sup> *Helv. Chim. Acta*, 1934, **17**, 726.

<sup>80</sup> *J. Amer. Chem. Soc.*, 1944, **66**, 1188.

<sup>81</sup> *Ibid.*, 1943, **65**, 424.

As a result of such pronounced concentration effects, ultracentrifuge boundaries become abnormally sharp,<sup>31, 78</sup> preventing the estimation of diffusion coefficients from boundary spreading. Gralen,<sup>82</sup> assuming diffusion to be absent, has, however, used boundary spreading to give qualitative information on the polydispersity of cellulose and its derivatives.

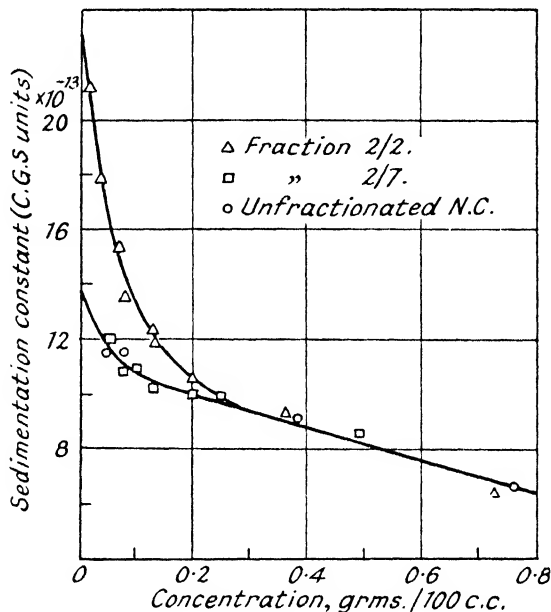


FIG. 4.

*Effect of concentration on the sedimentation constants of cellulose nitrates in acetone.*

(Reproduced by permission from Transactions of the Faraday Society, 1944, **40**, 229.)

The variation in sedimentation constant with concentration may justifiably be considered in terms of an increased effective frictional constant, arising from intermolecular interferences at higher concentrations, and leading invariably, therefore, to decreased sedimentation constants at higher concentration. In the case of diffusion, however, the situation is more complicated, since in addition to such variations in frictional constant, the "osmotic" driving force of diffusion is also non-ideal (with a value greater than ideal). Since the two factors are in opposition, their relative magnitudes decide whether the diffusion coefficient will increase, decrease, or remain unaffected with increasing solute concentration. For most linear polymers (especially the cellulose derivatives) the diffusion coefficient increases with concentration, apparently in a linear fashion (see p. 40).

In using equations (28) and (21) to calculate molecular weight and frictional constant, values of  $s$  and  $D$  at infinite dilution are clearly necessary. Both may theoretically be determined by extrapolation from low-concentration

<sup>82</sup> Ref. (16), p. 13.

measurements, but especially in the case of diffusion, where sufficiently low solute concentrations are not usually practicable, the extrapolation is not reliable. The more reliable procedure of using the Boltzmann equation to give a plot of diffusion coefficient against concentration for each experimental diagram of concentration or its gradient over the boundary region, has been performed by Gralen<sup>83</sup> for cellulose and its derivatives, and by Beckmann and Rosenberg<sup>24</sup> for a variety of polymers, who thereby confirmed that within the limits of experimental error the variation of diffusion coefficient with concentration is linear. Table VI contains sedimentation and diffusion data for certain cellulose nitrates in acetone with derived molecular weights, frictional and axial ratios, the latter based upon the assumptions of elongated ellipsoids of rotation and zero solvation.

TABLE VI.  
*Molecular weight and shape of cellulose nitrates in acetone.*

		(From Gralen. <sup>84</sup> )					
Nitrate of—		$\bar{s}_0$ $\times 10^{13}$ .	$D_0$ $\times 10^7$ .	$M$ $\times 10^{-3}$ .	$f/f_0$ .	$a/b$ .	
Unbleached	American						
linters	.....	19.0	1.00	780	12.2	870	11.3
Bleached	American						
linters	.....	14.0	1.44	400	10.6	670	9.8
Chlorite-bleached							
linters	.....	18.5	1.11	680	11.5	780	11.2
Sulphate cellulose	.....	16.2	1.56	420	9.6	550	10.7
Sulphite cellulose	.....	16.4	1.56	430	9.6	550	10.8
Holocellulose from							
spruce	.....	21.6	2.57	340	6.3	250	13.1

The last two columns contain the dimensions of the ellipsoidal model based upon the calculated axial ratio and molecular volume. In view of the assumptions involved in deriving these dimensions, a close analysis is not justifiable, but certain general conclusions can be drawn. Although the cellulose nitrate molecule in solution is very asymmetric, its axial ratio does not correspond to a completely stretched-out chain, unless the effective diameter of the chain be considerably increased by solvation<sup>78</sup> In view of the strong interaction between cellulose nitrate and acetone, this possibility is not unlikely. In the absence of quantitative information on solvation, it is impossible to decide between the alternatives of a solvated stretched chain and a folded unsolvated one. It is unfortunate that no check by other methods of the molecular weights and shapes of Table VI is available.

Several linear polymers have been investigated by the streaming birefringence method, which has included the study of the magnitude of the birefringence as well as the determination of rotational diffusion coefficients.<sup>36</sup> R. Signer and H. Gross<sup>85</sup> thus investigated polystyrenes and cellulose nitrates in solution, deriving rotational diffusion coefficients from which molecular dimensions can be estimated. Although some correlation of rotational

<sup>83</sup> Ref. (16), p. 59.

<sup>84</sup> Ref. (16), pp. 89, 97.

<sup>85</sup> *Z. physikal. Chem.*, 1933, A, **165**, 161.

diffusion coefficients and molecular weights, obtained viscometrically, was attempted for polystyrenes, it is only comparatively recently that more extensive correlations have been attempted. For instance, A. Wissler<sup>86</sup> investigated gelatine and its fractions, fractionated methyl cellulose and cellulose nitrate, and other polymers by the streaming birefringence method, and compared molecular dimensions so obtained with those from other methods. An extension of this valuable work is much to be desired.

Wissler made the interesting observation that the curves expressing the variation in corrected rotational diffusion coefficient ( $\Theta/\eta_{\text{solution}}$ ) or molecular length against degree of polymerisation were identical for methyl cellulose and cellulose nitrate, suggesting in agreement with equation (33) that molecular length, rather than thickness, controls rotational behaviour.

Interesting conclusions were reached by Signer and Gross<sup>85</sup> from birefringence measurements, and confirmed by R. Signer and C. Sadron.<sup>87</sup> For example, for the higher-molecular-weight polystyrenes, the curve of observed birefringence against velocity gradient, which is linear at low values of the gradient, suddenly assumes a higher value of the slope at a definite value of  $G\eta$ . A similar discontinuity also occurs at this point in the extinction angle, but neither effect was observed in cellulose nitrate solutions which, rather, showed signs of a saturation birefringence at high gradients. Signer therefore reasonably considered the discontinuity to be caused by the onset of stretching of the curled-up polystyrene chain, which clearly is expected to be absent in already extended chains of the cellulose type.

The flexibility of the normally coiled polymer chain in solution is also to be expected from the dielectric properties of solid linear polymers, for which the range of relaxation times observed can only be explained as due to local orientations of dipolar portions of the molecule, made possible by some free rotation within the molecule.

Some further confirmation of these views on molecular flexibility has been obtained from measurements of viscosity and light scattering. Thus, T. Alfrey, A. Bartovics, and H. Mark<sup>88</sup> showed that the intrinsic viscosity of solutions of polystyrene in good solvents was higher than in poor solvents, and could be lowered by the addition of precipitant, indicating a more compact configuration for the polymer molecule in the presence of a solvent with which it does not interact. Again, no such changes were observed with cellulose acetate solutions. Doty, Affens, and Zimm<sup>54</sup> have investigated these conclusions by calculating, from light-scattering measurements, the root-mean-square length of the polystyrene molecule in solution, assuming it to be a random coil. Table VII, containing the results of some of their calculations, demonstrates clearly the changes in molecular form occurring between different solvents.

For a change in molecular dimensions of 20%, Table VII indicates a change of 500% in intrinsic viscosity—a clear warning of the dangers involved

<sup>86</sup> Inaugural Thesis, Bern, 1940.

<sup>87</sup> *Helv. Chim. Acta*, 1936, **19**, 1324.

<sup>88</sup> *J. Amer. Chem. Soc.*, 1942, **64**, 1557.

TABLE VII.

*Molecular data on polystyrene fractions.*

Fraction.	Solvent.	Weight average mol. wt., $M_w$ .	Extended length, A.	Root-mean- square length.	$[\eta_{sp.}/c] \rightarrow 0$ .
RT-H	Toluene	4,800,000	115,000	2370	7.6
„	Ethylene dichloride	4,800,000	115,000	2390	5.3
„	Butanone	4,800,000	115,000	2100	2.3
„	Butanone- <i>isopropanol</i>	4,800,000	115,000	1900	1.6
BZO-4	Ethylene dichloride	2,100,000	50,000	1100	2.16
„	Butanone	2,100,000	50,000	1230	1.45
„	Butanone- <i>isopropanol</i>	2,100,000	50,000	990	0.84

in using viscosity alone as a measure of molecular weight or shape. Light-scattering measurements have also been used<sup>13</sup> to calculate the molecular weights of cellulose acetate fractions for which osmotic values were already known, an average agreement within 8% being recorded. From the observed dissymmetry of scattering, it was concluded, in agreement with other workers (*e.g.*, H. Mosimann;<sup>89</sup> N. Gralen<sup>90</sup>) on cellulose and its derivatives, that the lower-molecular-weight cellulose acetate molecules ( $M < 80,000$ ) are considerably extended, but that larger molecules coil back upon themselves. Polarisation measurements<sup>55</sup> confirmed this conclusion and indicated the more coiled state of the molecules of polyvinyl chloride and polystyrene.

From this review, it is evident that much valuable information, derived chiefly by the independent application of the different experimental methods, is now available. A beginning has also been made in comparing the differently achieved results for individual systems; much more work of a similar though more extensive character is, however, required before the available data, especially in their quantitative aspects, can be unreservedly accepted.

P. J.

### 3. CRYSTAL GROWTH.

There has been no report on crystal growth for some fifteen years. In view of the fact that many problems in this field are of such theoretical interest and of such importance in industry, the comparative neglect of the subject in this country is somewhat surprising. A bare recital of experimental results and of theories propounded would probably only serve to confirm the view that "differences of *opinion* such as expressed by the various workers studying crystal growth and adsorption phenomena strikingly emphasise the need for exact knowledge concerning the way in which a crystal grows".<sup>1</sup> From the apparently capricious behaviour of crystals one might justifiably gain the impression that crystal growth is not a subject for precise scientific study. A review of work in this field suggests, first, that there has in general been too much divorce of theory from experiment,

<sup>89</sup> *Helv. Chim. Acta*, 1943, **26**, 61.<sup>90</sup> *Ref. (16)*, p. 95.<sup>1</sup> W. G. France, "Colloid Chemistry," J. Alexander, Reinhold Publishing Corporation, New York, 1944, Vol. 5, p. 443.

and, secondly, that it is difficult to be sure what problem a particular piece of work was designed to solve. It is still true to say, as it was fifteen years ago,<sup>2</sup> "that it is almost impossible to draw any certain conclusions from the mass of information that has been collected on the growth and solution of crystals, because very few of the aspects have been investigated from all sides, and insufficient systematic work has been carried out". It is necessary first to decide what facts have been, or need to be, established, so that the problems can be precisely defined. We have to establish that under specified conditions growth takes place in a definite and reproducible way, and that certain variations in these conditions lead to reproducible variations in the mode of growth. The theoretical treatment can then make provision for all the relevant variables. A characteristic of much work on crystal growth, both theoretical and experimental, is that only special cases are dealt with, and these are sometimes complex rather than simple. Here we may make one general observation. The difficulty of growing single crystals with perfect geometrical shapes emphasises a complication inherent in all crystal-growth experiments. In a process involving isolated particles (*e.g.*, reactions in gases or liquids) the result always represents the average behaviour of a large number of reacting units, and the differences between the particles are governed by well-known distribution laws. Any abnormal behaviour on the part of a few molecules is taken care of in this way. In the growth of a single crystal, however, the position is rather different. If, for some accidental cause, the deposition of a particular layer of molecules departs from normal and if this is not soon corrected, the subsequent behaviour of an indefinite number of molecules may be entirely altered. Thus from a statistical standpoint the whole crystal corresponds to a single molecule in the gas reaction, so that data on large numbers of crystals are necessary before reproducible and significant results can be expected. This has to be borne in mind when assessing the value of observations on the growth of single crystals, for some of the most careful measurements have been made on a small number of crystals (even on one crystal). The time factor alone might make the extension of the experiment to, say, one hundred crystals an impracticable proposition.

This Report will be largely confined to crystal growth from solution, and it is convenient to consider this under three heads: (*a*) normal growth from pure solution, (*b*) growth with change of shape, and (*c*) growth from solutions containing impurities.

(*a*) *Growth from Pure Solution*.—Casual observations on crystals grown from the same solution would suggest that there can be variations not only in the relative areas of faces but even in the types of faces present. Three fundamental questions are therefore: (1) do crystals in fact maintain the same geometrical shape during growth from pure solution under certain specified conditions, (2) if so, is this shape dependent on the absolute rate of growth, and (3) how is the face development dependent on the nature

<sup>2</sup> *Ann. Reports*, 1931, 28, 279.



of the solvent? In spite of the obvious importance of (1) this point seems to have received very little study. It involves the measurement of the dimensions of crystals of various sizes, and the crystals must develop faces of two or more kinds during normal growth. Most of the precise studies of crystal growth have been concerned with substances such as sodium chloride, sodium chlorate, and alum, the normal developments of which comprise only one type of face (cube or octahedron). Points (2) and (3) will be discussed under (c).

(b) *Growth of a Crystal whose Shape is changing.*—If a salt such as sodium chloride is being studied, and it is desired to measure the rates of growth  $v_{hkl}$ , etc., perpendicular to faces of different types, it is necessary to produce faces such as (110) or (111) by some means other than normal growth from pure solution. Cases which have been studied in some detail include the growth of spheres of sodium chloride through intermediate shapes to cubes, the further growth of crystals from pure solution in solutions containing habit-modifying impurities, or *vice versa*. Here the problem is to account for the relative rates of growth on faces of two kinds, those which normally appear and those which would not be present (or, at any rate, would be present to different extents) in normal growth. Thus, cube faces may be grown on an alum crystal by adding a dye to the solution, and the further growth of this crystal with (100) and (111) faces may be studied in pure alum solution, from which the crystals normally grow with only octahedron faces. In such a case we require to determine whether  $v_{100}/v_{111}$  is constant—whether it depends on the relative developments of the forms {100} and {111}, on the absolute rate of growth, and so on. We return to this point later.

(c) *Growth from Solutions containing Impurities.*—The interpretation of relative growth rates on different faces of crystals grown in solutions containing impurities is likely to be extremely difficult. In the case of crystals exhibiting two or more forms grown from pure solution, it does not appear to be known whether the relative developments of the forms depend on the absolute rate of growth of the crystal. However, from solutions containing impurities the relative developments of different forms do definitely depend on the rate of growth. We may define the rate of growth either in terms of the mass of the whole crystal, which increases as some function of the time, or in terms of the rate of displacement of a face parallel to itself. If the latter were kept constant, and the crystal maintained the same shape, then the mass of the crystal would increase as the cube of the time, a condition difficult to achieve experimentally and apparently never attempted. In practice, there are two simple ways of controlling growth: (1) lowering the temperature of an initially saturated solution, or (2) removing the solvent at a constant rate at constant temperature. (It is assumed here that all solute is deposited on a specified number of seed crystals.) In either case, and assuming in (1) a linear solubility-temperature relation, the mass of the crystal increases linearly with time, *i.e.*, the rate of growth perpendicular to any face is continually decreasing. To the Reporter's knowledge no

detailed study has been made of the effect of the absolute rate of growth on the shape of crystals grown either from pure solution or from solutions containing impurities.

Any general theory of crystal growth from solution must take account of the solvent. In certain cases the importance of the solvent is obvious, as, for example, when change of solvent entirely alters the face development of the crystal. Iodoform crystallises from certain solvents as hexagonal bipyramids and from others as prisms terminated by the basal planes. In the case of resorcinol crystallising from water, the strong interaction with the solvent entirely prevents growth on the hydroxylic faces at one end of the crystal. In all cases, however, there must be some degree of interaction between solute and solvent (*a*) in the solution, and (*b*) at the crystal faces. Before a sodium ion can be attached to a growing crystal of sodium chloride it need only be partly dehydrated, for when the ion is attached to the crystal it may still be solvated on the side towards the solution. Before it is finally incorporated in the crystal, in a layer below the surface, this remaining solvent must be removed. If this separation of solvent is not complete, solvent becomes trapped in the crystal, and inclusions (of saturated solution) are common in crystals which have been grown rapidly. Thus the rapid advance of a crystal face implies a drift of solvent molecules away from the surface against the flow of solute towards it, for whatever the degree of supersaturation near the surface of the crystal the number of solute particles in unit volume of the solution is less than in the crystal. In the extreme case, when growth becomes very rapid, diffusion of solute from the bulk of the solution may become the rate-determining factor, and the normal relations between the relative rates of growth on different types of face become altered or destroyed.

There is clearly much in common between the parts played by the solvent and by a habit-modifying impurity in the process of crystal growth. Inasmuch as both impurity and solvent are adsorbed to varying degrees on crystal faces, it would seem that the action of adsorbed impurity and of solvent could be dealt with in a similar way in a generalised treatment of crystal growth from solution. We have noted that the interaction of solvent with certain crystal faces can be as specific, and as far-reaching in its effect, as that of some adsorbed impurities, and just as inclusions of solvent are formed in crystals grown very rapidly from pure solution, so a habit-modifying impurity may be trapped in a rapidly grown crystal. Since the shape of a crystal grown from a solution containing a habit-modifying impurity apparently does depend on the absolute rate of growth, it seems reasonable to suppose that this may also be true for growth from a pure solution.

Although it is impossible to draw a hard-and-fast line between the various aspects of crystal growth, we shall for convenience adopt the following plan: (1) theoretical, including relations between internal structure and face development, (2) studies of the growth of single crystals, (3) effect of impurities on crystal habit, (4) oriented overgrowths, (5) perfection of internal structure and of crystal faces, (6) miscellaneous topics, including

(a) supersaturation and nucleus formation and (b) technique of growing single crystals.

(1) *Theoretical*.—In the most general terms, we may say, with (Miss) M. Bentivoglio,<sup>3</sup> that the form of a crystal is influenced by three main factors: (a) its internal structure, (b) the nature of the solvent, and (c) a variety of external factors, not easily subjected to exact study, such as its proximity to the walls of the vessel or to other crystals, and the effect of diffusion or concentration gradients. "The first two factors conjoined may be expected to lead to a standard or ideal habit to which individuals tend to conform in so far as they are not modified by the disturbing influences of the third category. This ideal habit is that which a crystal would assume if it could be subjected to uniform conditions of growth from the first moment of its formation. (It can be obtained by constructing planes parallel to the various faces observed on the actual crystal at distances from a central point proportional to their measured rates of growth.)" Clearly, if we wished to account for the way in which a crystal would grow under any conditions (*e.g.*, in unstirred supersaturated solution) we should have to allow for external factors such as concentration gradients which we know can lead even to quite different rates of growth of identical faces. There is good reason to believe that the ideal habits of crystals are simple,<sup>4</sup> and that the usual illustrations of crystals do not represent what we may call the fundamental morphology of the substance. We shall not attempt here any general review of the numerous theories of crystal growth, but shall discuss two topics which have been the subject of a number of papers in recent years. The first represents one possible approach to the problem of accounting for the face development of a crystal; the second is purely crystallographic and takes account only of the internal structure of the crystal, *i.e.*, only the first of the three factors enumerated above.

*The concept of minimum total surface free energy.* The condition for stability of an isolated drop of a fluid is that its surface free energy, and hence its area, is a minimum. W. Gibbs<sup>5</sup> suggested that a similar condition might apply to a crystal, *viz.*, that for a crystal in equilibrium with its surroundings at constant temperature and pressure, its total (Gibbs) free energy is a minimum for a given volume. If we suppose that the volume free energy per unit volume is constant throughout the crystal, then the condition

becomes  $\sum_1^N A_i g_i = \text{minimum}$ , where  $g_i$  is the surface free energy per unit area of the  $i$ th face of area  $A_i$  on a crystal bounded by  $N$  faces. In other words, those faces develop which lead to a minimum total surface free energy for a given volume, so that any crystal if kept for a sufficiently long time in a saturated solution should attain the equilibrium shape determined by this criterion. Gibbs realised that, owing to the different degrees of internal order in crystals and liquids, the practical realisation of an equilibrated

<sup>3</sup> *Proc. Roy. Soc.*, 1927, A, 115, 59.    <sup>4</sup> A. F. Wells, *Phil. Mag.*, in the press.

<sup>5</sup> "Collected Works," Longmans, Vol. 1, p. 320.

crystal might be difficult. In a drop of liquid there is random arrangement of the molecules (or atoms) so that every portion of the surface is similar in structure to every other. In a crystal, however, the structural units are arranged in a regular way in three dimensions so that the whole array possesses certain symmetry. The bounding surfaces of the crystal are plane (see Section 5) but not necessarily equivalent, and there is an infinite series of sets of possible faces for any crystal. The growth of a crystal takes place by the deposition of new layers on the faces of the crystal, and there might, therefore, be activation energies associated, for example, with the commencement of a new layer, and these might be independent of the area of the face. Gibbs concluded that "it seems not improbable that the form of very minute crystals in equilibrium with solvents is principally determined by the condition that  $\sum_1^N (A_i g_i)$  shall be a minimum for the volume of the crystal but as they grow larger (in a solvent no more supersaturated than is necessary to make them grow at all), the deposition of new matter on the different surfaces will be determined more by the nature (orientation) of the surfaces and less by their size and relations to the surrounding surfaces. The *kinds* of surface thus determined will probably generally be those for which  $g_i$  has the least values. But the *relative developments* of the different kinds of sides will not be such as to make  $\sum_1^N (A_i g_i)$  a minimum."

G. Wulff<sup>6</sup> showed that the Gibbs equilibrium shape of a crystal is very simply related to the relative surface free energies of the faces. The crystal should form a polyhedron such that the perpendicular distances from a point within the crystal to the faces are proportional to the specific surface free energies of the appropriate faces. M. von Laue<sup>7</sup> has recently reviewed, and added to, the various proofs of this theorem, and emphasised that it is not sufficient merely to determine, for any arbitrary combination of faces, the shape corresponding to minimum surface free energy. It would be necessary to consider all possible combinations of faces and to find which of the relative minima is actually the smallest. The validity of the minimum surface free energy criterion could only be tested directly if the relative values of these energies of sufficient (ideally, of all the possible) faces of a crystal were known, and unfortunately very little is known of these quantities. Indirect evidence has been adduced to show that the Gibbs condition is not normally applicable to crystals grown at a finite rate. It is known, however, that the crystals of a given substance grown slowly from a particular solvent generally exhibit a simple development, which we may call the normal shape, and it has been shown that crystals of other shapes do tend, on further growth, towards the standard shape.<sup>3, 8</sup> Such changes do not, however, take place unless growth is permitted. For example, an octahedron of sodium chloride, grown from a solution containing urea, does not change

<sup>6</sup> *Z. Krist.*, 1901, **34**, 449.

<sup>7</sup> *Ibid.*, 1943, **105**, 124.

<sup>8</sup> A. F. Wells, *Phil. Mag.*, 1946, **37**, 184.

into a cube in pure solution at constant temperature. This fact indicates that the criterion of Gibbs cannot be applied in its original simple form to a macroscopic crystal which has been grown at a finite rate. An essential difference between a drop of fluid and a crystal is that the former can adjust its shape by the rearrangement, within itself, of its atoms or molecules. This is not generally possible at ordinary temperatures for a crystal. Provided no other crystals are present, a crystal of non-equilibrium shape in a saturated solution could rearrange itself to the equilibrium shape only by the passage of molecules either *via* the solution or through some surface layer from one face on to others, or on to other parts of the same face. Such processes do not take place at measurable rates in the case of macroscopic crystals, except possibly at temperatures near the melting point. In order to define the shape of a crystal we need to know what factors determine the relative growth rates on the different faces. The simple Gibbs picture would imply that these growth rates are directly proportional to the appropriate specific surface free energies. This is clearly not true for *any* absolute rate of growth of the crystal, for the Gibbs criterion is applicable to a crystal in equilibrium with its surroundings. It may well be, however, that the Gibbs equilibrated crystal is the limiting shape for infinitely slow growth. To the Reporter's knowledge, no attempt has yet been made to study crystals grown from solution under conditions such that the experiments would constitute a test of the Gibbs hypothesis.

Many contributions to the theory of the growth of crystals and the equilibrium shape of crystals have been made by Stranski and his co-workers, developing the Kossel-Stranski theory of crystal growth.<sup>9</sup> For example, a detailed theoretical study of the geometrical aspect of the growth of a crystal of sodium chloride has been given,<sup>10</sup> and an interpretation of the Thomson-Gibbs equation in terms of the work of separation of individual atoms.<sup>11, 12</sup> It is shown that if  $\phi_a$  is the separation work per atom averaged over an entire surface plane of the crystal, then an alternative statement of the Gibbs-Wulff theorem is that all faces on a crystal in equilibrium with the same vapour phase must have the same value of  $\phi_a$ . This work will not be dealt with further here as it is not very relevant to crystal growth from solution, but for completeness references are given below<sup>13, 14, 15</sup> to supplement those given in the earlier Report.<sup>9</sup>

*The relation between Bravais lattice, space-group, and face development.*  
In a crystal the structural units are arranged in conformity with one of the

<sup>9</sup> See *Ann. Reports*, 1931, **28**, 276.

<sup>10</sup> I. N. Stranski, *Z. physikal. Chem.*, 1932, **B**, **17**, 127.

<sup>11</sup> I. N. Stranski and R. Kaishev, *ibid.*, 1934, **B**, **26**, 100, 114, 312.

<sup>12</sup> *Idem*, *ibid.*, 1937, **B**, **35**, 427.

<sup>13</sup> I. N. Stranski *et al.*, *ibid.*, 1931, **B**, **11**, 342; *Kolloidchem. Beih.*, 1931, **32**, 197; *Z. Krist.*, 1931, **78**, 373; 1934, **88**, 325; 1943, **105**, 91; *Physikal. Z.*, 1935, **36**, 393; *Ann. Physik*, 1935, **23**, 330; *Ber. Wien. Akad.*, 1936, **145**, 840; 1938, **146**, 800.

<sup>14</sup> M. Volmer, *Ann. Physik*, 1935, **23**, 44; "Kinetik der Phasenbildung," Dresden, 1939, pp. 42, 87-97.

<sup>15</sup> W. Kossel, *Ann. Physik*, 1934, **21**, 457.

fourteen Bravais lattices. Bravais suggested that the face development of a crystal was closely related to its fundamental lattice, the faces being parallel to the net planes in the lattice and those planes most closely packed with lattice points being the most important forms on the crystal. G. Friedel<sup>16</sup> showed that this is often true. The Bravais "law" implies that the Miller indices of crystal faces will be small integers, and it is well known that faces with high indices are rare. It was not to be expected that the law would be universally valid, for it took account only of the internal structure of the crystal, and it was realised that external factors such as the rate of crystallisation and the presence of impurities in the solution could affect the habits of crystals. The Bravais law was elaborated by J. D. H. Donnay and D. Harker,<sup>17</sup> who showed that not only the type of lattice but also the presence of screw axes and glide planes (other than those inherent in the lattice) should be taken into account in correlating face development with internal structure. The Bravais law states that the importance of a form  $\{hkl\}$  is proportional to the reticular density (number of lattice points in unit area) or inversely proportional to the reticular area  $S_{hkl}$ . Since  $S_{hkl}d_{hkl} = V$ , where  $d_{hkl}$  is the spacing of successive planes  $hkl$  and  $V$  is the volume of the unit cell, the importance of a form should be directly proportional to the interplanar spacing  $d_{hkl}$ . Donnay pointed out that since screw axes and glide planes involve translations, their presence alters the effective spacings of planes perpendicular to the former and of certain planes perpendicular to the latter. For example, a  $3_1$  axis changes the effective spacing of the (0001) plane from  $c$  to  $c/3$ , and since this is the only plane affected, the importance of the form  $\{0001\}$  relative to others is altered. It is evident that the planes affected in this way are just those of which certain orders of reflexion of X-rays are absent owing to destructive interference (the systematic absences listed in space-group tables).

If the importance of a form  $\{hkl\}$  is proportional to the spacing  $d_{hkl}$  it is possible to draw up for any crystal a list of planes arranged in order of decreasing  $d_{hkl}$ , and the position of a given plane in this "morphological aspect" (M.A.) should express the importance of the form relative to others. The Donnay-Harker extension of the Bravais law requires that, instead of using in all cases the simplest Miller indices, we use the multiple indices of the lowest order of X-ray reflexion compatible with the space-group symmetry. In propounding the new law the authors claimed only that it was an improved approximation, for it is obviously subject to the same limitations as regards external factors as was the original law of Bravais. In addition, Donnay and Harker pointed out that if the space-group symmetry were the only influential factor then all cubic crystals, for example, with the same space-group would exhibit the same face development, whereas this is not so. A morphological aspect is defined as "a possible list of decreasingly important forms for any set of axial elements (axial

<sup>16</sup> *Compt. rend.*, 1904, **139**, 221, 314; *Bull. Soc. franç. Min.*, 1905, **28**, 6; 1907, **30**, 326.

<sup>17</sup> (a) *Compt. rend.*, 1937, **204**, 274; (b) *Amer. Min.*, 1937, **22**, 446.

ratios and interaxial angles) in any given crystal system". Corresponding to the 230 space-groups there are 97 morphological aspects.<sup>18</sup> Only in the cubic system can the planes be listed for a given M.A. without reference to a particular crystal; in other systems the axial elements affect the order of the planes. In order to determine the space-group from the face development the M.A. drawn up from observations on the crystals is compared with the above theoretical M.A.'s. In the case of minerals the experimental M.A. is drawn up in a standard way<sup>19</sup> by making a statistical survey of the frequency of occurrence and relative sizes of various forms on a large number of crystals from different localities. Donnay and others have shown that in a number of cases there is good agreement between the observed face development and that predicted from the space-group, and that in some cases the space-group could be deduced or confirmed from morphological studies.<sup>20</sup> Crystals studied in this way include rhombic sulphur, pyrites, spinel, and analcime,<sup>17b</sup> stephanite,<sup>21</sup> garnet,<sup>22</sup> muscovite,<sup>23</sup> scheelite,<sup>24</sup> goethite and lepidocrocite.<sup>25</sup>

This work of Donnay and others on the relation between the face developments and space-groups of crystals has been critically reviewed.<sup>26</sup> Apart from the fact that morphological relationships which do not take account of the solvent are necessarily only of limited validity, it is shown that there are other complications of a purely crystallographic nature which severely restrict the usefulness of the Donnay-Harker generalisation. The significance of the face developments (experimental M.A.'s) of natural crystals is also discussed in another paper.<sup>4</sup>

It is appropriate to mention here some cases of anomalous face developments. It has long been known that in certain cases etch-pits on crystals indicate a symmetry lower than that of the internal structure, or in some cases of the normal face development. For example, L. Royer<sup>27</sup> studied the etching of calcite by various acids and impure natural hydrocarbons, and concluded that the apparent low symmetry resulted from the nature of the etching medium. Equally interesting are the cases of crystals the number and arrangement of faces on which indicate a symmetry lower than that of the structure, *i.e.*, where the low symmetry results from growth instead of dissolution. Examples are lead and barium nitrates, assigned to class 23 on morphological evidence (space-group  $Pa3$ ), and cuprous oxide, ammonium and potassium chlorides, assigned to class 43 (space-groups  $Pn3m$ ,  $Pm3m$ , and  $Fm3m$  respectively). It seems likely that the

<sup>18</sup> J. D. H. Donnay and D. Harker, *Naturaliste canadien*, 1940, **67**, 33.

<sup>19</sup> J. A. Tremblay, *J. Washington Acad. Sci.*, 1942, **32**, 327.

<sup>20</sup> J. D. H. Donnay, *Amer. Min.*, 1938, **23**, 5; 1939, **24**, 184.

<sup>21</sup> E. D. Taylor, *ibid.*, 1940, **25**, 327.

<sup>22</sup> J. D. H. Donnay and C. Faessler, *Univ. Toronto Studies, Geol. Ser.*, 1941, **46**, 19.

<sup>23</sup> M. A. Peacock and R. B. Ferguson, *ibid.*, 1943, **48**, 65.

<sup>24</sup> J. D. H. Donnay, *Trans. Roy. Soc. Canada*, 1942, **36**, Sec. 4, 37.

<sup>25</sup> M. A. Peacock, *ibid.*, p. 107.

<sup>26</sup> A. F. Wells, *Phil. Mag.*, 1946, **37**, 217.

<sup>27</sup> *Compt. rend.*, 1929, **188**, 1176, 1303; 1929, **189**, 932; 1930, **190**, 503.

environment of the crystal during growth may be responsible for some of these anomalous developments, and the value of evidence such as the number and arrangement of faces of high indices is questionable.<sup>4</sup> The apparent hemihedrism of crystals of lead chloride, lead bromide, and mercuric bromide grown from solutions containing organic colloids provides a further example of this phenomenon.<sup>28</sup> W. Kleber<sup>29</sup> supposes the enantiomorphous development of crystals such as cuprous oxide (with a non-enantiomorphous structure) to be due to preferential adsorption of some optically active substance from the solution, melt or vapour.

(2) *The Growth of Single Crystals*.—The study of the growth in aqueous solution of spheres of sodium chloride cut from a single crystal, initiated by K. Spangenberg,<sup>30,31</sup> has been continued by a number of workers.<sup>32,33,34</sup> By using spheres cut from natural crystals it was found that the final shape was always a cube. The growth of crystals with cube and octahedron faces was also studied, both in pure solution and solutions containing various added salts or urea. A summary of this work is available.<sup>35</sup> The study has been extended to a number of other alkali halides,<sup>36</sup> the single crystals being grown from the melt by the Kyropoulos method. The earlier results for sodium chloride were confirmed, and it was shown that in the case of potassium bromide and potassium iodide also the final product would be a cube. From the measurements on the chloride, however, Morgenstern concluded that the final form would be an octahedron, a surprising result for which no explanation is suggested. Differences were also observed in the forms present at intermediate stages during growth. For example, potassium bromide, alone among the halides studied, never showed octahedron faces. Some preliminary results on the development of vicinal faces on sodium chloride crystals during growth have been reported by K. Spangenberg,<sup>37</sup> who points out that no significant differences in growth of natural or artificial crystals can be detected.

From his own experiments on the growth of single crystals France<sup>1</sup> concludes that "the theories of Niggli, Valetton, and Spangenberg are generally acceptable". It seems difficult, however, to reconcile this statement with France's own experimental results. J. J. P. Valetton<sup>38</sup> assigned to each type of face a specific growth velocity (solely dependent on its ionic structure and independent of its size and the sizes of adjacent faces), and supposed that the rate of growth on a face was determined by (a) its specific growth velocity and (b) the speed at which ions can diffuse to the face. P. A. Paine and W. G. France<sup>39</sup> measured  $v_{100}/v_{111}$  for potassium alum and

<sup>28</sup> F. D. Miles, *Proc. Roy. Soc.*, 1931, A, **132**, 266. <sup>29</sup> *Naturwiss.*, 1944, **32**, 77.

<sup>30</sup> F. Gille and K. Spangenberg, *Z. Krist.*, 1927, **65**, 204.

<sup>31</sup> K. Spangenberg, *Jahrb. Min.*, 1928, **57**, 123.

<sup>32</sup> W. Schnorr, *Z. Krist.*, 1928, **68**, 1.

<sup>33</sup> A. Neuhaus, *ibid.*, p. 10.

<sup>34</sup> E. Ernst, *ibid.*, 1937, **96**, 38.

<sup>35</sup> K. Spangenberg, "Handwörterbuch der Naturwiss.," Jena, 2nd ed., 1934, **10**, 372.

<sup>36</sup> H. Morgenstern, *Z. Krist.*, 1938, **100**, 221.

<sup>37</sup> *Ibid.*, p. 82.

<sup>38</sup> *Ibid.*, 1924, **59**, 135, 335.

<sup>39</sup> *J. Physical Chem.*, 1935, **39**, 425.



found that it varied with the degree of stirring, increasing from 1.61 in unstirred to 1.75 in stirred solution. This they explained as due to the increased rate of diffusion, though it is not clear why this should favour  $v_{100}$  rather than, for example, increase both  $v_{100}$  and  $v_{111}$ . The explanation of the results for ammonium alum appears even more unsatisfactory. G. W. Bennett and W. G. France<sup>40</sup> determined  $v_{100}/v_{111}$  as 1.53 for ammonium alum growing in pure solution, under conditions of constant humidity. (They state that the normal form of the crystals is an octahedron with small cube faces.) M. E. Lash and W. G. France<sup>41</sup> then grew small cubes of the salt in a solution containing a dye and measured  $v_{100}/v_{111}$  for further growth in pure solution. They obtained the value 2.32, though it is not stated how constant this ratio remained with time. The following statements are then made: "It might be expected that this ratio would be different if instead of a normal crystal with octahedral faces present, a seed crystal having only cube faces was used. Due to the large area of the cube faces compared to that of the octahedral faces, the velocity of their perpendicular displacement should be greater. . . . This more rapid growth of the cube faces is to be expected on the basis of the theory of crystal growth advanced by Niggli.<sup>42</sup> The cube face of the alum crystal has a higher growth velocity than the octahedral: hence if the cube faces are the only ones present, as in the case of the cubic crystal, their perpendicular growth will be more rapid than normal until they are reduced to their usual area or disappear entirely." Here France accepts the view that  $v_{100}/v_{111}$  is *not* a constant for ammonium alum but depends on the relative areas of cube and octahedral faces present. This is clearly not consistent with the simple theory of Valetton, and suggests that it would be worth while to measure this ratio for crystals with different proportions of cube and octahedron faces, and under well-defined conditions of growth, in order to see exactly what factors do determine its value.

The above results are also not in agreement with the earlier findings of Bentivoglio,<sup>3</sup> who grew crystals of various double sulphates and neutral tartrates and found: (1) that similar faces of a simple form grow at the same rate, *even when of different sizes* (hence a misshapen crystal, such as a flattened octahedron or cube, if grown larger under uniform conditions tends towards but never attains the ideal shape with equal faces), and (2) if faces of more than one type are present they grow at different rates which are characteristic of the different kinds of face. It was also concluded that like faces grow at the same rate except when adjacent to a large face of another fast-growing form, which causes impoverishment of the solution in its neighbourhood and destroys the uniformity of the conditions. It would seem that Bentivoglio's experiments were on the right lines in that the crystals studied were those which in normal growth develop faces of more than one type. It is apparently still not known whether  $v_{hkl}$  is in

<sup>40</sup> J. Amer. Ceramic Soc., 1928 **11**, 571.

<sup>41</sup> J. Physical Chem., 1930, **34**, 724.

<sup>42</sup> P. Niggli, Z. anorg. Chem., 1920, **110**, 55.

fact a constant independent of the area of (*hkl*). In Bentivoglio's experiments a saturated solution was simply allowed to cool in a uncontrolled way, and growth was fairly rapid.

W. Nernst<sup>43</sup> supposed the rate of growth of a crystal to be governed solely by diffusion processes, the solution in contact with the crystal being exactly saturated. H. A. Miers,<sup>44</sup> however, actually measured the concentration of solute in the solution in contact with a face of a growing crystal of sodium chlorate, using the crystal itself as a prism, and showed that the solution was definitely supersaturated. In the later theories of crystal growth some relation has generally been assumed between rate of growth and degree of supersaturation of the solution near the crystal face. A more recent study<sup>45</sup> goes a stage further in measuring the variation in the degree of supersaturation over a single face of a growing crystal. Briefly the method is as follows. A thin platy crystal, surrounded by supersaturated solution, is enclosed between two half-silvered glass plates forming a wedge of very small angle. The faces to be studied are approximately normal to the mirrors. This system is illuminated by parallel monochromatic light at normal incidence, interference fringes being formed. These fringes are straight if the light has passed through the solution far from the crystal, but are curved in the vicinity of the crystal, owing to changes in the refractive index of the solution around the growing crystal. The fringes are photographed at intervals and from the deviations of the fringes from straight lines it is possible to calculate the refractive index of the solution at any point. From a knowledge of the way in which the refractive index varies with concentration, the concentration of the solution at any point around the crystal can be calculated. It was found that the concentration varies across a crystal face, being least at its centre. Even at the centre of the face the solution was still supersaturated to a small degree. Berg attempted a detailed analysis of the concentration gradients over a crystal face and concluded that the supply of solute arriving at the face from the solution was deficient near the edges of the face so that, since the face does remain plane, movement of solute in an adsorbed layer across the face must take place. It seems open to question whether studies of growth under these conditions (gradual exhaustion of a supersaturated solution) are the most satisfactory line of attack. In some of Berg's experiments, for example, faces of the same crystallographic type on the same crystal, which were in contact with solution of the same degree of supersaturation, grew at different rates. This anomalous behaviour was attributed to the presence of traces of impurity. A very small alteration in the concentration gradients around a crystal growing in stagnant supersaturated solution is sufficient to alter the mode of growth of a crystal in a radical way. A more fundamental objection to this type of experiment is perhaps the fact that the observed concentration gradients are the *result* of the crystal growing in the way it

<sup>43</sup> *Z. physikal. Chem.*, 1904, **47**, 52.

<sup>44</sup> *Phil. Trans.*, 1904, **202**, 459.

<sup>45</sup> W. F. Berg, *Proc. Roy. Soc.*, 1938, **A**, **164**, 79.

does (*i.e.*, at different rates in different directions) so it is difficult to see exactly what information can be obtained from the experiment. The importance of eliminating diffusion effects is illustrated by the experiments of A. Papapetrou<sup>46</sup> on dendrites of potassium chloride. The low concentration at the centres of the cube faces gave rise first to "inverse vicinal faces" and then to dendritic growths growing out from each corner of the cube.

(3) *The Effect of Impurities on Crystal Habit.*—A considerable amount of work has been done on the effect of dyes on the habits of crystals. The experimental fact is that a large number of dyes of the most diverse types, when added to solutions of certain inorganic salts in very small quantities, produce striking changes in the habits of the crystals growing from the solutions. References to the earlier work are given by H. E. Buckley and W. Cocker.<sup>47</sup> Recent work in this field has been reported in two series of papers, by Buckley in this country and by France in America. The work of H. E. Buckley<sup>48</sup> was qualitative, in the sense that crystals of the salts were grown from solutions containing different amounts of dye and the habit-modifying efficacy was assessed from visual estimates of the relative importance of forms of different kinds. In a recent review<sup>49</sup> of his work Buckley attempts to correlate the properties of the dye molecules with their habit-modifying power. From his results on oxy-salts such as potassium sulphate, chlorate, and perchlorate, he concluded that any large molecule containing a  $\text{SO}_3^-$  group, if this is not *ortho* to a diazonium group or occupying the *peri*-position in a naphthalene nucleus, will function as a habit-modifier. The  $\text{COO}^-$  and  $\text{OH}$  groups are less effective, and the effect of dyes like Bismarck-brown and methylene-blue seems to be specific to a few crystals only. When there are two (or more)  $\text{SO}_3^-$  groups in the same molecule the effect depends on their relative positions. In some cases there appears to be a rough correlation between the overall size of the dye molecule and its efficiency in causing habit changes. In view of the complex structures of these dye molecules it seems unlikely that any detailed interpretation of these results is at present possible, particularly as the "habit-modifying power" is not a well-defined measured quantity. A further complication is the complex nature of dye solutions. Studies of absorption spectra of methylene-blue solutions indicate that they contain mixtures of various polymers, particularly at higher concentrations.<sup>50</sup>

France and his co-workers<sup>51</sup> have made extensive studies of habit modification by impurities in aqueous solution, and France has published

<sup>46</sup> *Z. Krist.*, 1935, **92**, 89..

<sup>47</sup> *Ibid.*, 1933, 858.

<sup>48</sup> *Ibid.*, 1930, **73**, 443; **75**, 15; **76**, 147; 1931, **78**, 412; **80**, 238; 1932, **81**, 157; **82**, 31, 285; 1934, **88**, 122, 248, 381; 1935, **91**, 375; 1937, **97**, 370.

<sup>49</sup> *Proc. Manchester Lit. Phil. Soc.*, 1939, **83**, 31.

<sup>50</sup> T. Vickerstaff and D. R. Lemin, *Nature*, 1946, **157**, 373.

<sup>51</sup> *J. Amer. Ceramic Soc.*, 1927, **10**, 435, 579, 821; 1928, **11**, 571; *J. Amer. Chem. Soc.*, 1924, **46**, 540; 1941, **63**, 1505; *Coll. Symp. Annual*, 1925, **3**, 317; 1930, **7**, 59; *J. Physical Chem.*, 1930, **34**, 724, 2236; 1932, **36**, 2832; 1935, **39**, 425; 1936, **40**, 81, 177; 1938, **42**, 1079; 1941, **45**, 395; 1942, **46**, 1044.

a review of this work.<sup>1</sup> This has been mainly concerned with the mechanism of adsorption of foreign substances at the crystal-solution interface during growth and with the habit changes resulting from such adsorption. He has attempted to discover both how the adsorbed particles are held on the crystal and the nature of the forces responsible for the adsorption. Experimental methods used include (a) the measurement of changes in growth ratios due to adsorption, (b) the correlation of crystal structure with habit modification, (c) X-ray and electron-diffraction studies of crystals containing dyes that have modified the habit, (d) the determination of the quantity of dye adsorbed by growing crystals, and (e) the effect of the molecular structure of the dyes on their adsorption by crystals. The work was started on potassium and ammonium alums and later extended to sodium, barium, lead, and lithium nitrates, sodium bromate, potassium chloride, bromide, and sulphate, sodium sulphate, cupric acetate, urea, and citric and tartaric acids. Some of this work is qualitative and some quantitative, a photographic record of the growth of the single crystal being used to determine the relative growth rates on the different faces. Some of these measurements have been discussed in Section 2. France concludes, rather comprehensively, that the adsorption of a foreign substance by a growing crystal is dependent on (a) the lattice structure of the host crystal, (b) the residual valency force fields, (c) the ionic structure of the crystal face, and (d) the presence, size, shape, position, and orientation of polar groups within the foreign molecule. As regards the detailed behaviour of particular dyes, however, there does not appear to be complete agreement between different workers.

C. Frondel<sup>52</sup> has studied the effect of 112 dyes on the habits and optics of LiF, NaF, NaCl, KCl, KBr, and KI. He found that there was adsorption on (111) in a few cases, but in general if the dye was adsorbed at all it was adsorbed on (100). He concludes that the mechanism suggested by Buckley for habit modification of crystalline oxy-salts by dyes cannot apply here, in view of the lack of common structural units in the halide and dye. Also, the adsorption on cube faces (with equal numbers of positive and negative ions) rather than on octahedron faces is unexpected, but no explanations are suggested. This paper contains a very useful list, with references, of all recorded cases of change of habit caused by an impurity in solution. Frondel<sup>53</sup> has also investigated the effect of 143 impurities on the habit of sodium fluoride crystals grown from aqueous solution. He places the salts in two classes. Those which provide hydroxyl ions (NaOH, Na<sub>2</sub>CO<sub>3</sub>, NaCN) are said to function owing to the substitutional adsorption of hydroxyl for fluoride ions in the surface of the growing crystal. Other salts, such as Na<sub>3</sub>PO<sub>4</sub>, which form crystalline double salts with sodium fluoride, are supposed to form a "two-dimensional, essentially crystalline double-salt phase" on the surface of the sodium fluoride crystal. The habit change is then attributed to the fact that the double-salt formation takes place pre-

<sup>52</sup> *Amer. Min.*, 1940, **25**, 91.

<sup>53</sup> *Ibid.*, p. 338.

ferentially on those planes of the sodium fluoride crystal which are structurally related to some plane in the crystalline salt (compare the views of Bunn and Royer, Section 4).

F. D. Miles<sup>54</sup> studied the effect of colloidal materials (dextrin and gum arabic) and some dyes on the crystallisation of lead chloride, bromide, iodide, and azide. Points of interest include the formation of sphenoidal crystals of the chloride, attributed to the selective effect of the dextrin on one of the two sets of holohedral faces, leading to hemihedral development, and of spherulitic growths at higher dextrin concentrations. Considerable amounts of colloid are adsorbed by lead azide and other salts, up to 10% by weight in the case of lead azide. Apparently up to 4—5% of dextrin can be taken up by crystals which still appear to be single crystals when examined microscopically, but higher concentrations give spherulitic aggregates. X-Ray examination showed that the lattice spacings of the salts are unaffected by the introduction of the colloid, and it was concluded that the crystals were skeletal growths of crystallites, in nearly the same orientation, with layers of colloid between them. The influence of dextrin<sup>54</sup> or gelatin<sup>55, 56</sup> on the habit of lead iodide is negligible. This salt always forms thin hexagonal plates, though these colloids certainly retard nucleus formation. In the case of lead azide the  $\alpha$ -form is normally precipitated in aqueous solution, but if 0.02% of eosin is present the lead azide is precipitated in the  $\beta$ -form.

The habit of ammonium dihydrogen phosphate crystals is modified by the addition to the solution of various ions ( $\text{Sn}^{4+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{3+}$ , etc.)<sup>57</sup>. The normal habit is the tetragonal prism {100} terminated by {101}. These ions prevent deposition on the prism faces and cause replacement of the terminal pyramid faces by tapering "faces", the angle of taper varying, for a given foreign ion, with its concentration. At high concentrations of foreign ion, growth on the seed crystal stops completely. An attempt was made to correlate the degree of tapering with the solubility product of the metallic hydroxide, data on the phosphate solubilities being scarce. The problem is, however, more complex, for the degree of tapering depends also on the degree of agitation of the solution and on the rate of growth of the crystals. Moreover, any simple explanation in terms of adsorption cannot account for tapering, because the total surface area of a tapered crystal is the same as that of a larger crystal of normal shape, *i.e.*, the impurity has not altered the relative areas of prism and pyramid faces, as is usually the case with adsorbed impurities, but the way in which the layers terminate. In a preliminary study of the modification of the habit of ammonium oxalate monohydrate by foreign ions, tapered crystals were also obtained.<sup>58</sup> The effect of foreign ions ( $\text{Fe}^{3+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ , etc.) on the habits of ammonium

<sup>54</sup> *Phil. Trans.*, 1935, A, **235**, 125.

<sup>55</sup> T. P. Bolam, *Trans. Faraday Soc.*, 1930, **26**, 133.

<sup>56</sup> T. P. Bolam and W. J. Donaldson, *ibid.*, 1933, **29**, 864.

<sup>57</sup> H. J. Kolb and J. J. Comer, *J. Amer. Chem. Soc.*, 1945, **67**, 894.

<sup>58</sup> *Idem, ibid.*, 1946, **68**, 719.

chloride and bromide has been studied by J. J. Tilmans.<sup>59</sup> According to K. N. Ozerov,<sup>60</sup> the habits of natural corundum crystals can be related to the type of rock (acidic or basic) with which they were associated.

(4) *Oriented Overgrowths*.—The formation of oriented overgrowths of one crystal on another has always been of interest in connection with isomorphism. If we describe as isomorphous crystals with the same number and arrangement of atoms in the unit cell (*e.g.*, all compounds with the rock-salt structure) then it is clear that further conditions must be satisfied if the crystals are to form (a) solid solutions, or (b) oriented overgrowths. For solid-solution formation there must be fairly close correspondence between interatomic distances in the two structures and also the chemical properties of the substances must be similar. [We should, perhaps, remark that solid-solution formation does not necessarily imply that the components are isomorphous—witness  $\text{CaF}_2$  and  $\text{YF}_3$ ,  $\text{AgBr}$  (rock-salt structure) and  $\text{AgI}$  (zinc-blende structure).] The conditions for the formation of oriented overgrowths are less stringent. If two isomorphous compounds are very similar chemically and the size factors are very favourable, as in the case of potassium aluminium and potassium chromium alums, complete overgrowths of one component on the other are possible. In the case of calcite and sodium nitrate, solid solutions are not formed but oriented (isolated) crystals of the latter may readily be grown on a fresh cleavage surface of calcite. These are examples of overgrowths of isomorphous substances. However, compounds with quite different structures can form oriented overgrowths. Thus if in the crystals of oxy-compounds the arrangement of the large oxygen ions is similar in certain planes of the two crystals, the structures of which are in other respects quite different, then crystals of the one substance may grow on certain faces of a crystal of the other (compare the growth of sodium nitrate on mica, Section 6). More striking examples were given by L. Royer,<sup>61</sup> who showed that whereas non-ionising substances such as naphthalene and anthracene would not form oriented overgrowths on crystals of rock-salt, lead sulphide, calcite, or mica, yet organic compounds which can ionise in solution can form such overgrowths. For instance, quinol (hexagonal modification) and *pp'*-dihydroxydiphenyl<sup>62</sup> grow as oriented crystals on calcite or sodium nitrate. A two-dimensional correspondence between the two crystal structures in certain planes is usually supposed to be a necessary condition for such overgrowths, though little is known of the detailed structures of many of these complex organic compounds.<sup>63, 64</sup>

Many examples of oriented overgrowths have been discovered or re-studied in systematic studies carried out in recent years, of which the following are typical. Alizarin and other anthraquinone derivatives form oriented

<sup>59</sup> *J. Gen. Chem. Russia.*, 1941, **11**, 869.

<sup>60</sup> *Compt. rend. Acad. Sci. U.R.S.S.*, 1945, **47**, 49.

<sup>61</sup> *Compt. rend.*, 1933, **196**, 709.

<sup>62</sup> J. Willems, *Z. Krist.*, 1938, **100**, 272.

<sup>63</sup> H. Seifert, *Fortschr. Min.*, 1936, **20**, 324.

<sup>64</sup> *Idem*, *Z. Krist.*, 1937, **96**, 111.

overgrowths on crystals of zinc, antimony, and lead sulphides,<sup>65</sup> and attempts have been made to relate the molecular structure of the organic material to the structure of the supporting crystal. Alizarin also forms oriented overgrowths on (100) of sodium chloride and other alkali halides, (10 $\bar{1}$ 1) of sodium nitrate and calcite, (11 $\bar{2}$ 1) of sodium nitrate, (111) of fluorspar, and (001) of mica.<sup>66</sup> Oriented overgrowths of  $\beta$ -silver iodide on silver bromide have been produced.<sup>67</sup> Thiourea and urea form similar oriented overgrowths on mica, and the former grows in an oriented manner on zinc sulphide, sodium chloride, and antimony sulphide. Urea does not form oriented overgrowths on these sulphides.<sup>68</sup> Bromanil orients on metallic silver (100) and on sodium chloride (100),<sup>69</sup> while chloranil and anthracene form regular intergrowths.<sup>70</sup>

A number of workers have emphasised the close connection between adsorption of impurities on crystal faces, leading to modification of habit, and the formation of oriented overgrowths and mixed crystals.<sup>71, 72</sup> Under appropriate conditions an impurity may be incorporated into the body of the crystal, as in the case of the birefringent cubes of sodium chlorate containing thiosulphate, or of ammonium chloride containing urea. These "adsorption bodies" are generally unstable, and the impurity gradually migrates out of the crystal, a phenomenon also observed with some "super-saturated" mixed crystals. C. W. Bunn supposed that there would be greatest modification of crystal habit where there is very strong similarity of specific lattice planes but complete dissimilarity of the rest of the structures, and supported the argument by showing a metrical correspondence between the atomic arrangement in, for example, a (100) face of ammonium chloride and (001) of urea, and by showing that oriented overgrowths of urea could be formed on cube faces of ammonium chloride. Although there is obviously a connection between the formation of oriented overgrowths and habit modification, it seems unlikely that this static picture requiring correspondence between the atomic arrangement in certain planes of the host crystal and of the *crystalline* habit-modifying impurity is true for all the numerous cases of habit modification. Adsorption of the impurity as isolated ions or molecules would appear to be sufficient to affect the rate of deposition on a crystal face, and provided the average population of adsorbed particles on one face at any time is different from that on another, modification of habit is to be expected. As soon as a *crystal* of the adsorbed substance is formed (overgrowth) then interatomic distances in the crystalline impurity become relevant. It is by no means certain, however, that exact correspondence of the two structures in one plane is necessary, for we may imagine the overgrowth anchored to the substrate

<sup>65</sup> A. Neuhaus, *Naturwiss.*, 1943, **31**, 387.

<sup>66</sup> *Idem*, *Z. physikal. Chem.*, 1943, **A**, **192**, 309.

<sup>67</sup> G. M. Schwab, *Naturwiss.*, 1943, **31**, 322.

<sup>68</sup> A. Neuhaus, *ibid.*, 1944, **32**, 34.

<sup>69</sup> A. Neuhaus and W. Noll, *ibid.*, p. 76.

<sup>70</sup> J. Willems, *Ber.*, 1944, **77**, 17.

<sup>71</sup> P. Gaubert, *Compt. rend.*, 1918, **167**, 491; 1925, **180**, 378; 1932, **194**, 109.

<sup>72</sup> C. W. Bunn, *Proc. Roy. Soc.*, 1933, **A**, **141**, 567.

only along lines, or even at points. In any case, the picture of two crystals fitting perfectly together over a plane surface of union is probably far from the truth, as will be evident from the remarks in the next section on the probable structure of actual crystal faces.

(5) *The Perfection of Internal Structure and Faces of Crystals.*—The concept of the mosaic structure of crystals was introduced by Darwin in 1914, since when it has been discussed in a large number of papers, particularly in the *Zeitschrift für Kristallographie* (1934, 89). Although a detailed discussion of mosaic structure is not possible here, it is relevant to consider its origin in terms of the mechanism of crystal growth. A difference between the lattice parameters in the surface layers of a crystal and in the interior (the Lennard-Jones effect) was postulated by F. Zwicky<sup>73</sup> and later calculated by J. E. Lennard-Jones and B. M. Dent<sup>74</sup>. As the size of a crystal is reduced the volume of the external layers increases in proportion to the volume of the whole crystal, so that for very small crystals it becomes justifiable to speak of the parameter of the crystal as a whole, this being a mean value. It was shown<sup>75</sup> that the parameter of very small crystals must be greater than normal in the case of ionic crystals but less than normal for homopolar crystals. This effect has been verified experimentally for certain metals (nickel,<sup>76</sup> copper and iron<sup>77</sup>). As a crystal grows, the external layers become internal ones and in the process the interatomic distances therefore require readjustment. This readjustment can only go to completion if the mean thermal energy of the atoms or ions of the lattice is sufficient to enable them to adopt their equilibrium positions. If this does not happen, however, the deviation of the structure from the ideal cannot continue indefinitely. At some time the formation of a new nucleus will be more advantageous from the energy standpoint, and therefore more likely, than the further growth of the existing crystal with the deformed lattice. Thus the growth of the first crystal ceases and the new unstrained crystal begins to grow, i.e., the crystal growth is intermittent and leads to a structure consisting of separate (mosaic) blocks. The intermittent nature of the crystallisation of metallic crystals has been confirmed in the case of crystals grown from the vapour<sup>78</sup> and deposited electrolytically. Photomicrographs of electrolytically deposited nickel,<sup>79, 80</sup> cobalt,<sup>81</sup> copper,<sup>82</sup> etc., show a layer structure, the individual layers having a thickness of around  $10^{-4}$  cm. According to R. Suhrmann and H. Schnackenberg,<sup>83</sup> the

<sup>73</sup> *Physikal. Z.*, 1923, **24**, 131.

<sup>74</sup> *Proc. Roy. Soc.*, 1928, **A**, **121**, 347.

<sup>75</sup> J. E. Lennard-Jones, *Z. Krist.*, 1930, **79**, 215.

<sup>76</sup> H. Boochs, *Ann. Physik*, 1939, **35**, 333.

<sup>77</sup> N. A. Shishakov, *J. Exptl. Theoret. Physics U.S.S.R.*, 1940, **10**, 1450.

<sup>78</sup> M. Straumanis, *Z. physikal. Chem.*, 1931, **B**, **13**, 316; 1932, **B**, **19**, 63.

<sup>79</sup> A. W. Hotherhall and G. E. Gardam, *Metal Ind., London*, 1939, **55**, Nos. 21 and

22.

<sup>80</sup> E. Raub and M. Wittum, *Z. Elektrochem.*, 1940, **46**, 71.

<sup>81</sup> G. A. Moore, *Trans. Electrochem. Soc.*, 1937, **71**, 247.

<sup>82</sup> V. Mattacotti, *Metal Ind., N.Y.*, 1939, **37**, 259.

<sup>83</sup> *Z. Elektrochem.*, 1941, **47**, 277.



energy of activation  $U$  of the ordering process (the readjustment mentioned above) ranges from 150 cal./g.-atom for bismuth to 800 cal./g.-atom for nickel, and is very much less than for processes involving change of place of atoms in the crystal. The number of atoms  $n$  capable of passing from states of non-equilibrium to states of equilibrium, when their total number is  $N$ , will be given by  $n = Ne^{-U/kT}$ . If crystallisation takes place at a temperature  $T \gg U/k$  (the temperature of rest) then deformation of the lattice should not occur. It is also necessary that the rate of crystallisation be not greater than the rate of the ordering process. M. Renninger<sup>84</sup> showed that in crystals grown from the melt by the Kyropoulos method there is no pronounced mosaic structure, in contrast to natural crystals of rock-salt.

V. S. Joffé<sup>85</sup> distinguished three types of stress in real crystals. Stresses of the third kind arise, and are counterbalanced, within a particular mosaic block, owing to the difference between the parameters of the internal layers and the surface layers. Stresses of the second kind arise because contiguous external layers of neighbouring blocks have different parameters, for the plane at which the growth of one crystal block finishes (hence that farthest removed from the state of equilibrium) touches the plane at which growth of the next block commences (hence the plane nearest to the state of equilibrium). These stresses are also counterbalanced locally. Just as a single mosaic block is built up of layers, so the whole crystal is built up of mosaic blocks, and Joffé supposes that if  $a_0$  is the parameter of the internal layers and  $a_2$  that of the free surface of the crystal, then the parameter  $a_1$  of the internal surface of the crystal will have some value intermediate between  $a_0$  and  $a_2$ . For an ionic crystal  $a_0 > a_1 > a_2$ , and for a homopolar or metallic crystal  $a_0 < a_1 < a_2$ . By analogy with the stresses of the third kind there should arise stresses of the first kind, counterbalanced within the limits of volumes comparable with the volume of the whole crystal. These stresses would be oriented and capable, on reaching a certain magnitude, of causing splitting or disruption of the crystal. Hence, on reaching a certain size a mosaic crystal should become mechanically unstable, and disruption of large crystals grown from solution has been observed.<sup>86</sup> It would therefore be of some interest, as has been pointed out elsewhere in another connection,<sup>87, 88, 89</sup> to have information about the largest known crystals of different substances, particularly if the maximum size could be correlated with the mode of growth and degree of mosaic structure. It is interesting that in a number of cases it has been shown<sup>90</sup> that if an impurity is added to the solution much larger crystals can be grown. Joffé suggests that many of the abnormal types of crystal growth should be explicable in terms of his picture of "real" crystals. A detailed description of many types of

<sup>84</sup> *Z. Krist.*, 1934, **89**, 344.

<sup>85</sup> *Uspekhi Khimii*, 1944, **13**, 144.

<sup>86</sup> A. V. Shubnikov, "How Crystals Grow," Moscow, 1935.

<sup>87</sup> C. Palache, *Amer. Min.*, 1932, **17**, 362.

<sup>88</sup> C. Frondel, *ibid.*, 1935, **20**, 469.

<sup>89</sup> J. W. Retgers, *Z. physikal. Chem.*, 1892, **9**, 278.

<sup>90</sup> W. E. Gibbs and W. Clayton, *Nature*, 1924, **113**, 492.

abnormality has been given by D. B. Gogoberidze.<sup>91</sup> According to F. Bernauer<sup>92</sup> the bifoliate type of spherulite arises by growth in the direction of the long axis of the crystal at a constant rate accompanied by splitting at a constant angular velocity.

In the above picture a real crystal, grown at too low a temperature, is in a metastable condition and not in true thermodynamic equilibrium. In contrast to this, D. Balarew<sup>93</sup> has developed a theory of "growth conglomerates", based on two postulates: first, that a crystal with perfect surfaces and possessing edges and corners can never be in thermodynamic equilibrium with its environment, and second, that a large perfect crystal must pass over spontaneously into one with mosaic structure. He supposes that definite conditions of crystallisation give rise to a particular crystalline conglomerate and that the intermittent growth of crystals leads to a growth conglomerate comprising separate layers which is in thermodynamic equilibrium. The theory appears to be founded on a misinterpretation of the Thomson-Gibbs equation as applied to crystals. For a liquid, this equation, viz.,  $p_r = p_\infty e^{2\gamma\sigma/rR^2}$ , relates the vapour pressure of a droplet of radius  $r$  to the surface free energy per unit area ( $\sigma$ ), molecular volume ( $V$ ), and the vapour pressure of a plane liquid surface. When this is applied to a crystal,  $r$  becomes the central distance of the plane face (distance from the "Wulff point"), but Balarew<sup>94</sup> assumes it to mean the radius of curvature and regards corners and edges as having very great curvature, "of atomic dimensions". The theory that crystals grow or dissolve "block by block" seems to rest on very doubtful experimental evidence. For example, in one of his experiments Balarew claims to show that the solubility of gypsum depends on the *direction* of the stirring.<sup>95</sup> As I. N. Stranski<sup>96</sup> points out, in a critical review of Balarew's work, it is difficult to imagine how the forces between blocks of the size suggested (some  $10^{-4}$  cm. side) could be adequate to orient the blocks in the formation of a single crystal, and moreover the mode of formation of the blocks has not been explained. Further references are given to Balarew's work.<sup>97</sup>

The shape of a crystal may, for many purposes, be described as a convex polyhedron, and it was the perfection of many crystal faces which made possible the development of crystallography as a science. However, there are many ways in which crystals depart from the above description. First, there are the cases in which the crystal is still essentially a convex polyhedron but (a) the simple faces are replaced by vicinal faces, (b) the "face" is actually formed into ridges caused by the alternation of faces of two types,

<sup>91</sup> *Usp. Fiz. Nauk.*, 1940, **2**, 242.

<sup>92</sup> "Gedrillte Kristalle", Berlin, 1929.

<sup>93</sup> "Der disperse Bau der festen Systeme", Dresden, 1939.

<sup>94</sup> *Kolloidchem. Beih.*, 1930, **30**, 258; 1931, **32**, 203; 1933, **37**, 184; *Z. Krist.*, 1934, **89**, 268; 1936, **93**, 166.

<sup>95</sup> D. Balarew and N. Kolarew, *ibid.*, 1939, **101**, 156.

<sup>96</sup> *Ibid.*, 1943, **105**, 91.

<sup>97</sup> D. Balarew, *ibid.*, 1938, **100**, 167; *Zentr. Min.*, 1941, 228; *Kolloidchem. Beih.*, 1939, **50**, 178; 1940, **51**, 123; 1941, **52**, 45; *Kolloid-Z.*, 1939, **88**, 161, 268; 1940, **92**, 82; 1942, **98**, 43.

(c) the crystal is tapered, (d) the whole face is curved, or (e) there are localised imperfections on an otherwise plane face. The last three phenomena appear, at least in many cases, to be associated with the presence of impurities in the solution, as, for example, in the case of the low conical hillocks on (011) faces of potassium perchlorate crystals grown in the presence of certain dyes.<sup>98</sup> The same phenomenon may be observed on the tetrahedron faces of sodium chlorate crystals grown from solutions containing sodium thio-sulphate. In the second large group come the more radical departures from normal growth as a single crystal, such as spherulitic, dendritic, and twinned structures. If a solution of sodium carbonate is allowed to diffuse slowly into a gel containing barium chloride (prepared from commercial gelatin and allowed to set) the precipitation of the barium carbonate takes place in layers, and a variety of crystal forms is observed. These range from needles, through "sheaves" to spherical aggregates. The structures and optical properties of these spherulites have been studied recently in some detail, particularly by H. W. Morse and his co-workers.<sup>99</sup> The growth of the practically spherical aggregates and of the intermediate forms can be accounted for if it is postulated that crystallisation radiates from a central nucleus in a limited solid angle only. It is assumed that growth is fastest in one direction, that every point at the surface of the growing cone of fibres can act as a new starting point for further radiating growth, and that the spatial extent of the latter is controlled by the possible angle of aperture of the cone and by the mechanical obstruction of the existing fibres. As growth proceeds, new fibres radiate from points reached by those formed earlier. The sheaf then opens out in fan-like manner until an approximately spherical shape is reached, when presumably the process stops owing to exhaustion of material in the environment. A somewhat similar explanation has been given<sup>100</sup> for the two-dimensional spherulites of substances such as malonamide and resorcinol grown between glass plates. Between crossed Nicols the three-dimensional spherulites show in parallel light an interference figure similar to that of a uniaxial crystal cut perpendicular to the optic axis and viewed in convergent light.

In considering the genesis of twin crystals, which he classifies into growth twins, transformation twins and gliding twins, M. J. Buerger<sup>1</sup> emphasises the close relation of twinning to polymorphism, and suggests that growth twinning is more likely, other things being equal, the greater the degree of supersaturation. Thus the condition causing supersaturation twins is most likely to arise just once—as the crystal nucleus forms—and not again, so that nuclei supersaturation twins are often characteristically simple pairs. This simple treatment does not account for the extraordinarily regular structure of some lamellar twins, such as those of potassium chlorate grown

<sup>98</sup> H. E. Buckley, *Z. Krist.*, 1934, **89**, 221.

<sup>99</sup> *Bull. Soc. franç. Min.*, 1931, **54**, 19; *Amer. J. Sci.*, 1932, **23**, 421, 440; 1933, **25**, 494; *Amer. Min.*, 1933, **18**, 66; 1936, **21**, 391.

<sup>100</sup> B. Popoff, *Latv. Farm. Zurn.*, 1934, 1.

<sup>1</sup> *Amer. Min.*, 1945, **30**, 469.

from supersaturated solution. To quote R. W. Wood,<sup>2</sup> "a plate which starts with twin planes 0.0002 mm. apart apparently builds up seven hundred laminae of the same thickness, while another plate starting with a different 'grating constant' sticks to it to the end." In other words, after a distance corresponding to some 300 unit cells, the orientation of the crystal changes, and this change takes place regularly at intervals of about 2000 Å.

In a paper on the surface motion of particles in crystals and the natural roughness of crystal faces J. Frenkel<sup>3</sup> begins by pointing out that vicinal faces with very high indices are not to be regarded as planes of high specific surface free energy (compare Miers's paradox,<sup>44</sup> that the faces actually present on a growing crystal of alum are those with very low densities of atoms per unit area), as is often assumed to be the case. In fact they consist of steps, the flat portions of which are planes of low indices [*e.g.*, (111) in the case of the vicinal faces on alum]. For the two-dimensional analogue, a staircase-like line with identical steps  $n$  units in length and 1 unit in height, the additional free energy per unit length is simply  $Nw$ , where  $w$  is the additional energy per step and  $N = 1/an = (1/a)\tan\phi$ ,  $a$  being the lattice constant and  $\phi$  the angle of inclination of the vicinal face to the basic face. Thus  $\sigma = \sigma_0 + wN = \sigma_0 + (w/a)\tan\phi$ . Since the surface free energy of the vicinal face is only slightly greater than that of the basic face it follows that the surface of a crystal in statistical equilibrium consists, not of a plane surface, but of a series of vicinal faces which arise spontaneously as the result of thermal fluctuations. This fluctuating roughness can be characterised by the ratio  $\lambda/a$  where  $\lambda$  is the mean length of the separate steps. Assuming  $\lambda \gg a$ , it is found that  $\lambda/a = \frac{1}{2}e^{w/kT}$ . To account for the variations in the areas of the terraces it is supposed that atoms can move freely over the horizontal portion of each terrace without interaction one with another. With respect to the "plane gas phase" adsorbed on a given terrace, the next terrace, lying at a higher level, plays the rôle of the condensed phase, and there exists a continuous exchange of atoms between the two plane phases, leading to fluctuations in the areas of the separate terraces. This concept is further extended to the edges of the terraces, the atoms linearly adsorbed on the rectilinear portion of each edge behaving as a kind of linear gas, so ensuring the possibility of a reshaping of the outlines of the separate terraces without changing their areas. The growth of a crystal is visualised as taking place by the random deposition of particles on the growing face, in general on the flat portions of the atomic terraces, thereby passing into the two-dimensional gas phase. Later, some of them become attached, still in a perfectly random way, to the vertical steps bounding the terraces (passing thus into the one-dimensional gas phase), and they move along until they become firmly attached at an angle (corner), as in the Kossel picture. This generalisation of the Kossel-Stranski theory is also applicable to vaporisation, dissolution or melting, when the above processes take place in the reverse order. A mechanism of this type has

<sup>2</sup> *Phil. Mag.*, 1909, **18**, 535.

<sup>3</sup> *J. Physics, U.S.S.R.*, 1945, **9**, 392.

been suggested by P. Lukirsky<sup>4</sup> to account for the development of vestigial crystal faces on the surface of a crystalline body ground initially in the form of a sphere, and subjected to more or less prolonged heating.

It might at first sight appear that certain observations on the movement of layers across the faces of growing crystals are in conflict with the above picture of crystal growth. Observations of the interference colours of thin crystals of *m*-toluidine<sup>5, 6</sup> indicate layers only a few molecules thick. The layers mentioned by C. W. Bunn and H. Emmett<sup>7</sup> must have a thickness of the order of the wave-length of visible light (some  $10^3$  atoms thick). They are observed only towards the edges of faces and presumably are the result of thin layers overtaking one another. Observations have also been made on layers spreading across faces of growing crystals of alkali halides,<sup>8</sup> and interpreted as supporting the Kossel-Stranski theory of the growth of ionic crystals. M. Volmer<sup>9</sup> has commented on the interpretation of some of these experiments. It seems likely that the above effects are observed only under conditions (*e.g.*, of rapid growth from supersaturated solutions) such that external factors—concentration gradients and diffusion effects—are important, and that they are not relevant to the case of a crystal growing slowly in a well-stirred solution. The former conditions, and also the presence of suitable impurities in certain cases, are known to lead to the formation of vicinal or curved faces, or tapered crystals. In all these cases the surface is not a normal face but the contour of the edges of layers, and the different types of divergence from normal plane faces of low indices represent different relations between the rate of spread of layers and the frequency of initiation of new layers.

S. Tolansky<sup>10</sup> has studied the topography of crystal faces by means of a multiple beam interferometric method. A highly reflecting film of silver about 500 Å. thick is deposited on the crystal face, which is placed near, and parallel to, an optical flat of quartz. Interference fringes are produced using a parallel beam of monochromatic light at normal incidence, and they show many interesting features of the structure of the crystal face. Examination of a (100) face of quartz, of high optical quality, showed the face to consist—not of a simple plane surface—but of vicinal faces inclined at angles varying from 0.50 to 9.00 minutes of arc and mostly curved, with radii of curvature from 20 to 60 metres. There were also sub-microscopic tetrahedral projections about 450 Å. high, which may represent nuclei from which subsequent growth would have started. A study of cleavage surfaces of mica and selenite<sup>11, 12</sup> showed steps on the surface of the former down to 40 Å., all the steps being multiples of 20 Å., the *c* spacing of mica as determined by X-ray diffraction. These steps are presumably the same as those in-

<sup>4</sup> *Compt. rend. Acad. Sci. U.R.S.S.*, 1945, **46**, 300.

<sup>5</sup> R. Marcelin, *Ann. Physique*, 1918, **10**, 185.

<sup>6</sup> L. Kowarski, *J. Chim. physique*, 1935, **32**, 303, 395, 469.

<sup>7</sup> *Nature*, 1946, **158**, 164.

<sup>8</sup> Z. Gyulai, *Z. Krist.*, 1935, **91**, 142.

<sup>9</sup> "Kinetik der Phasenbildung," p. 55.

<sup>10</sup> *Proc. Roy. Soc.*, 1945, **A**, **184**, 41.

<sup>11</sup> S. Tolansky, *ibid.*, p. 51.

<sup>12</sup> *Idem, ibid.*, 1946, **A**, **186**, 261.

ferred to exist on some mica surfaces from experiments made by Friedel on the orientation of ammonium iodide crystals on such surfaces.

(6) *Miscellaneous*.—(a) *Nucleus formation*. It is not possible to review here all the work done in the last few years on supersaturation and nucleus formation. The early work of Miers and others appeared to support Ostwald's view that at a given temperature there is a definite concentration below which crystals are not formed spontaneously (it being possible to maintain the solution indefinitely in this metastable state), whereas at higher concentrations spontaneous crystallisation occurs. The experiments of Miers, from which the actual "supersolubility" curve, between the metastable and labile regions, was plotted, only show that under the conditions of these experiments there was a fairly sharp boundary between the concentrations at which nucleus formation took place rapidly or fairly slowly. Later work showed that the area of the "metastable" region can be reduced by increasingly vigorous stirring or by the presence of foreign solid particles. Comparable results were obtained with melts, though in some cases if the rate of cooling is very great nuclei are not formed but a glass results. Although far less importance would now be attached to the precise position of Miers's supersolubility curve, since this has been shown to depend on the experimental conditions, it is generally agreed that just below the saturation point there is a region in which the probability of nucleus formation is small ("metastable" region), but that this probability increases rapidly beyond a certain degree of supersaturation. For a super-cooled liquid L. C. de Coppet<sup>13</sup> gave a simple kinetic explanation.

In technical crystallisation processes it is important to avoid excessive formation of nuclei on cooling surfaces. Rapid agitation does not overcome this difficulty as mechanical shocks cause nucleation in the body of the solution. One way in which this has been overcome is to carry out the supersaturation in one part of the apparatus and to allow crystal growth to take place in another vessel containing seed crystals, as in the Oslo crystalliser.<sup>14, 15</sup> The supersaturation of the solution travelling from the evaporator is insufficient for appreciable nucleus formation to take place but, of course, sufficient to cause growth of the seed crystals in the crystallising compartment. A kinetic derivation of the rate of nucleus formation from the vapour state has been given by I. N. Stranski and R. Kaishev.<sup>16</sup> Nucleus formation in supersaturated solutions can apparently be very capricious. For example, it was found in some experiments that by introducing seed crystals at the saturation temperature and then cooling, growth first occurred only on the seed crystals, then at a lower temperature a few new nuclei appeared, but only at a still lower temperature did nuclei form in large numbers throughout the solution.<sup>17</sup> Such effects are, however,

<sup>13</sup> *Ann. Chim. Phys.*, 1907, **10**, 457.

<sup>14</sup> F. Jeremiassen and H. Svanoe, *Chem. Met. Eng.*, 1932, **39**, 594.

<sup>15</sup> H. Svanoe, *Ind. Eng. Chem.*, 1940, **32**, 636.

<sup>16</sup> *Z. physikal. Chem.*, 1934, **B**, **28**, 317.

<sup>17</sup> H. H. Ting and W. L. McCabe, *Ind. Eng. Chem.*, 1934, **26**, 100.

very dependent on the size and total number of seed crystals, rate of cooling, speed of stirring, etc. Some recent papers on supersaturation and nucleus formation in solution are noted.<sup>18</sup> Brief reference only can be made to other recent work on crystallisation or recrystallisation processes. From studies of the kinetics of the crystallisation of sucrose solutions, A. van Hook<sup>19</sup> concludes that the rate of growth of the crystals is determined primarily by some interfacial reaction rather than an interboundary reaction, *i.e.*, that processes occurring at the crystal face (orientation and incorporation of molecules into the crystal) are more important than diffusion under the conditions of his experiments. The effect of added impurities was also studied.<sup>20</sup> The rate of crystallisation from supersaturated solutions of sodium sulphate has been studied.<sup>21</sup> According to W. Lotmar,<sup>22</sup> thin films of antimony deposited in a vacuum are originally amorphous, and crystallise spontaneously only if the film thickness exceeds a certain critical value. The growth of crystals during electrodeposition is considered in a theoretical paper by K. M. Gorbunova and P. D. Dankov,<sup>23</sup> and the growth of crystallites in supercooled liquid thymol by G. G. Jaemlein.<sup>24</sup> P. Laurent<sup>25</sup> has derived formulæ for the number of nuclei formed at a given time and for the velocity of crystallisation in allotropic transformations. The crystallisation of salts from thin films of solutions spread on mercury has been investigated by H. Devaux.<sup>26</sup>

(b) *Technique of growing single crystals.* There has been a number of papers concerned with the technique of growing large single crystals, as opposed to studies of the way in which the crystals grow (Section 2). They describe modifications of well-known methods. In order to obtain large crystals (up to 200 g.) of Rochelle salt with preferential development along the *y* and *z* axes, crystals may be grown between glass plates in a solution which is cooled from 30° at the rate of  $\frac{1}{2}$ —1° per day.<sup>27</sup> Large crystals of potassium dihydrogen phosphate<sup>28</sup> and alkali halides<sup>29</sup> may also be grown from aqueous solution. Single crystals of lithium fluoride, potassium bromide, and sodium chloride weighing up to 35 lbs. have been made<sup>30</sup> by melting the salt in a conical platinum crucible which is removed very slowly from the furnace into a lower cooler chamber, the crystal growing from the

<sup>18</sup> K. Neumann and A. Micss, *Ann. Physik*, 1942, **41**, 319; R. Gopal, *J. Indian Chem. Soc.*, 1944, **21**, 103, 145; B. S. Srikantan, *ibid.*, 1945, **22**, 55; O. M. Tode, *Acta Physicochim. U.R.S.S.*, 1940, **13**, 617; J. Amsler and P. Scherrer, *Helv. Physica Acta*, 1941, **14**, 318; C. G. Dunn, *Physical Rev.*, 1944, **66**, 215.

<sup>19</sup> *Ind. Eng. Chem.*, 1944, **36**, 1042, 1048; 1945, **37**, 782.

<sup>20</sup> A. van Hook, *ibid.*, 1946, **38**, 50.

<sup>21</sup> E. L. Krichevskaya, *J. Physical Chem. U.S.S.R.*, 1945, **19**, 382.

<sup>22</sup> *Helv. Physica Acta*, 1945, **18**, 232, 369.

<sup>23</sup> *Compt. rend. Acad. Sci. U.R.S.S.*, 1945, **48**, 15.

<sup>24</sup> *Ibid.*, p. 168.

<sup>25</sup> *Compt. rend.*, 1944, **219**, 205; *Rev. met.*, 1945, **42**, 22.

<sup>26</sup> *Compt. rend.*, 1944, **219**, 565.

<sup>27</sup> L. C. Baker, *New Zealand J. Sci. Tech.*, 1943, **25**, B, 62.

<sup>28</sup> W. Bantle, *Helv. Physica Acta*, 1943, **16**, 207.

<sup>29</sup> F. Henroteau, *Astronom. J.*, 1945, **51**, 122.

<sup>30</sup> R. L. Taylor and H. C. Kremers, *Chem. and Ind.*, 1944, **55**, 906.

end of the conical crucible. By allowing the crystallisation of the melt to start at a surface of a mica sheet, C. D. West<sup>31</sup> has obtained oriented sections of single crystals of sodium nitrate. The method is also applicable to sodium, potassium, and rubidium iodides and potassium bromide, when the crystal grows with (111) parallel to (001) of the mica. A modification of the original Verneuil furnace, in which the powdered material is projected into an oxy-hydrogen flame, has been used to obtain synthetic sapphires (single crystals of  $\alpha$ -alumina).<sup>32</sup> Fused silica may be converted into perfect small crystals of quartz when heated in a solution of sodium metasilicate.<sup>33</sup> Mixed thallous bromiodide single crystals have been prepared<sup>34</sup> for use in military infra-red optical instruments. Crystals containing 42 moles % of thallous bromide were grown from the melt by using a modified Bridgman furnace.<sup>35</sup> The melt was held at 470° in a furnace divided into two parts by an insulating baffle, the temperatures in the two sections being independently controlled, and the baffle serving to produce a steep temperature gradient in the region where growth took place. A conical crucible was used. The best results were obtained with a high temperature gradient and a slow rate of passage through the gradient. Methods of obtaining single crystals, particularly of metals, have been reviewed by A. Duran<sup>36</sup> (references to 32 papers). The first general method consists in slow cooling of the molten material in a crucible, either by removing the crucible slowly from the furnace (a method used by P. W. Bridgman<sup>37</sup> to obtain single crystals of W, Sb, Bi, Te, Cd, Zn and Sn, and recently by D. C. Stockbarger for lithium fluoride<sup>35</sup>) or by slowly cooling the whole furnace. Various devices are adopted to start the crystallisation from a nucleus with the desired orientation,<sup>38, 39</sup> and many designs of furnace and crucible have been developed.<sup>40</sup> The second method is to bring a nucleus into contact with the surface of the molten material and to withdraw the crystal slowly,<sup>41</sup> a method particularly useful for growing large single crystals of certain halides. A third method, recrystallisation in the solid state, has long been used for preparing mono-crystal wires of metals. Heating combined with compression in a steel mould has also been used.<sup>42</sup>

A. F. W.

<sup>31</sup> *J. Opt. Soc. Amer.*, 1945, **35**, 26.

<sup>32</sup> K. W. Brown, R. C. Chirnside, L. A. Dauncey, and H. P. Rooksby, *Gen. Electric (G.E.C.) Journal*, 1944, **13**, 53.

<sup>33</sup> N. Wooster and W. A. Wooster, *Nature*, 1946, **157**, 297.

<sup>34</sup> O. F. Tuttle and P. H. Egli, *J. Chem. Physics*, 1946, **14**, 571.

<sup>35</sup> *Rev. Sci. Instr.*, 1936, **7**, 133.

<sup>36</sup> *Anal. Fis. Quím.*, 1941, **37**, Supplement, p. 33.

<sup>37</sup> *Proc. Amer. Acad.*, 1925, **60**, 305.

<sup>38</sup> L. Graf, *Z. Physik*, 1931, **67**, 388.

<sup>39</sup> L. Schubnikow, *Proc. K. Akad. Wetensch. Amsterdam*, 1930, **33**, 327.

<sup>40</sup> H. Tazaki, *J. Sci. Hiroshima Univ.*, 1940, **A**, **10**, 37, 109; H. E. Farnsworth, *Physical Rev.*, 1935, **48**, 972; M. F. Hasler, *Rev. Sci. Instr.*, 1933, **4**, 656; C. A. Cinnamon, *ibid.*, 1934, **5**, 187.

<sup>41</sup> S. Kyropoulos, *Z. anorg. Chem.*, 1926, **154**, 308.

<sup>42</sup> H. S. Müller, *Z. Physik*, 1935, **96**, 321.



## 4. CRYSTALLOGRAPHY.

X-Ray diffraction by crystals is being widely applied to a great variety of problems. While the general stereochemical arrangements in molecules of some complexity such as penicillin and sucrose are examined and the less completely ordered structures of polymers or soap are studied, precise interatomic distances are determined in simpler substances such as methylammonium chloride. Some crystal structures such as that of ice which might be considered simple continue to reveal more and more detail as fuller use is made of all the observable X-ray effects. A mass attack has been made on the crystal chemistry of the rare earths, thorium, plutonium, neptunium and, it is presumed, other transuranic elements. As a result of the examination of 150 compounds it was claimed (at the Institute of Physics Conference on X-ray analysis during the War) that the crystal chemistry of these elements is now "known" better than that of most other elements. So far this knowledge is not available in detail. The structures of some complex chlorides of molybdenum have been revealed, but that there are still difficulties in structure determination is shown by work on  $\text{CsCuCl}_3$ <sup>1</sup>. This apparently simple structure seems to be based on close packing of caesium and chlorine ions, but no detailed arrangement has yet been found in agreement with the observed diffraction effects.

X-Ray examinations continue in use for identification, molecular-weight determination, and the testing of proposed molecular formulæ.<sup>2</sup> As an example of identification, the structure determinations of the plutonium and neptunium compounds mentioned above are of some interest. The chemical identities of most of the compounds were in this case deduced from their X-ray diffraction patterns given by very small quantities of materials prepared by known methods. The power of the X-ray method to reveal details that are with difficulty determinable by analytical methods is shown in a group of compounds that might have been supposed to be impure  $\text{Bi}_2\text{O}_3$  but which are shown to be built up of approximately spherical units of composition  $\text{SiBi}_{12}\text{O}_{20}$ <sup>3</sup> containing always an atom of silicon at the centre of the group. In another instance<sup>57</sup> the completion of a Fourier electron-density projection made possible by the existence of several related structures led to the revelation of a previously unsuspected molecule of methyl alcohol in the substance formerly known as  $\beta$ -quinol but thus shown and subsequently confirmed by analysis to be a compound of composition  $3\text{C}_6\text{H}_4(\text{OH})_2, \text{MeOH}$ . Weissenberg photography has been used to show that a sample presumed to be DDT had a deficiency of one chlorine atom per molecule and to identify it as DDD.<sup>4</sup>

<sup>1</sup> H. P. Klug and G. W. Sears, *J. Amer. Chem. Soc.*, 1946, **68**, 1133.

<sup>2</sup> E. P. Abraham, D. M. Crowfoot, A. E. Joseph, and E. M. Osborn, *Nature*, 1946, **158**, 744.

<sup>3</sup> L. G. Sillen; reported at Institute of Physics Conference on War-time Progress in X-Ray Analysis, July, 1946; see also *Arkiv Kemi, Min. Geol.*, 1937, **A**, **12**, 18. *Nature*, 1945, **155**, 305.

<sup>4</sup> M. Schneider and I. Fankuchen, *J. Amer. Chem. Soc.*, 1946, **68**, 2669.

B. Strijk and C. H. MacGillavry<sup>5</sup> have examined the structure of a high-temperature modification of sodium nitrite with a view to discover whether an abrupt change in the temperature coefficients of the cell constants and a simultaneous loss of the original strong piezoelectric effect may be due to the occurrence of two symmetrical sets of atomic positions in an average structure or to an oscillation of the atoms along one axis. A correction now given shows that a decision between the two models is not possible from the available data.

D. A. Hutchinson<sup>6</sup> has used density and X-ray data of calcite, diamond, lithium fluoride, sodium chloride, and potassium chloride to obtain atomic weights. This is done by comparing the molecular weights of two substances calculated from unit cell dimensions and densities. If the atomic weights of some of the elements are assumed, those of others, here calcium and fluorine, may be calculated. The values derived are  $\text{Ca} = 40.0849 \pm 0.003$ ,  $\text{F} = 18.9967 \pm 0.0013$  and it is concluded that such a determination is as reliable as other standard atomic weight procedures.

Other uses of X-rays include a study of the thermal decomposition of silver oxalate by means of oscillation and Weissenberg photographs.<sup>7</sup> The crystals are shown to undergo fragmentation in which portions of the original crystals break away and assume orientations in which their  $a$  axes are not parallel to that of the parent crystal. On further heating, the powder lines of metallic silver appear with definite maxima. This is presumably due to the orienting influence of the silver oxalate crystals. Many similar reactions could be investigated in this way.

*Experimental Methods and Calculations.*—A. Turner-Jones and C. W. Bunn<sup>8</sup> have extended the "tilted crystal" method of indexing, and describe a method of indexing the reflexions on rotation photographs of a single crystal set up in a random orientation. By this method it is possible to take an irregular fragment of a crystal of completely unknown crystallography, set it up on an ordinary X-ray rotation goniometer in any position, take two photographs, and deduce the unit cell and space-group from these photographs. M. Farquhar and H. Lipson<sup>9</sup> have discussed the general principles by which improved accuracy may be obtained in the determination of unit-cell dimensions from single-crystal photographs. The principles, based on those used for powder photographs, applied to an orthorhombic crystal enabled an accuracy of the order of 0.005% to be attained. The integral breadths of Debye-Scherrer lines for a divergent incident X-ray beam have been considered by A. J. C. Wilson.<sup>10</sup> The broadening due to the appreciable phase differences between different parts of the crystal, even in the size range for which line broadening occurs, is calculated and is shown to be ordinarily negligible.

The accuracy of atomic co-ordinates derived from X-ray data has been

<sup>5</sup> *Rec. Trav. chim.*, 1943, **62**, 705; 1946, **65**, 127.

<sup>6</sup> *J. Chim. Physics*, 1945, **13**, 383.

<sup>7</sup> R. L. Griffith, *ibid.*, 1946, **14**, 408.

<sup>8</sup> *J. Sci. Instr.*, 1946, **23**, 177.

<sup>9</sup> *Proc. Physical Soc.*, 1946, **58**, 200.

<sup>10</sup> *Ibid.*, p. 401.

the subject of theoretical consideration by A. D. Booth.<sup>11</sup> By a comparison of two independent sets of observed  $F$  values it is found that the error in experimentally observed  $F$ 's is independent of the magnitude of  $F$ . It is concluded that this source of inaccuracy in derived atomic co-ordinates is a secondary one and for a particular case the error for a carbon atom is estimated as approximately  $\pm 0.003$  Å. The larger error due to the non-infinite limits of Fourier summations is also considered and a possible way of correcting for it is devised. In a special case the distortion produced is found to have an upper limit between 0.02 and 0.005 Å., experimentally observed errors being about 0.02. The effect of thermal agitation is to give a considerable decrease in accuracy. Another paper<sup>11</sup> deals with the problem of determining the maxima in a Fourier synthesis, and is based on examining the rapidly varying differential coefficient rather than the function itself.

The problem of the steadily increasing magnitude of routine calculation necessary for any structure determination has received further attention. For computing Fourier series punched-card methods have been used with existing calculating machines by P. A. Schaffer, V. Schomaker, and L. Pauling,<sup>12</sup> who point out that the method has applications in other fields of molecular structure determination. Punched cards and a computing service were employed in the evaluation of electron densities for penicillin<sup>13</sup> at intervals of about 0.25 Å. throughout the unit cell. At present this procedure seems costly. Several machines for performing these calculations have been designed or constructed. One described by D. MacLachlan<sup>14</sup> depends on the spreading of layers of sand in sinusoidal waves over a scale plan of the unit cell so that the height of sand at any point is proportional to the electron density. An electrical Fourier summation machine has been developed by G. Hägg and T. Laurent.<sup>15</sup>

A. R. Stokes<sup>16</sup> has described a development of the "fly's eye" which obviates the calculation of structure amplitudes in the trial-and-error stages of structure determinations. In the device described, a regular repeated pattern representing the structure is produced on a photographic plate by use of a fly's eye composed of an array of small lenses embossed on the surface of a piece of "Perspex" which has been pressed at its softening temperature into a copper plate previously indented by means of a steel ballbearing. The disadvantages of the pin-hole method previously used, *viz.*, diffuseness of the pin-hole images and blocking of the pin-holes by dust, are overcome. Instead of a movable lamp to represent the atoms, a uniformly illuminated screen with a number of opaque discs may be used, so that no negative need be made and only one exposure is necessary instead of one for each atom.

As is well known, the determination of crystal structure by Fourier synthesis requires the observation of as many X-ray reflections as possible

<sup>11</sup> *Proc. Roy. Soc.*, 1946, **A**, 188, 77; *Trans. Faraday Soc.*, 1946, **43**, 444.

<sup>12</sup> *J. Chem. Physics*, 1946, **14**, 648.

<sup>13</sup> D. M. Crowfoot and B. W. Rogers, to be published.

<sup>14</sup> See *Nature*, 1946, **158**, 260.

<sup>15</sup> *J. Sci. Instr.*, 1946, **23**, 155.

<sup>16</sup> *Proc. Physical Soc.*, 1946, **58**, 306.

and the summation of appropriate Fourier series in which the structure factors, derived easily from these observations, appear as the coefficients. The obstacle to a simple and automatic application of the procedure to any desired crystal rests in the fact that the structure factor is a complex quantity. The magnitude is derivable from observed quantities but the phase angle escapes observation. At present all the essential work of the determination is that of discovering these phase angles by a process of trial greatly assisted by a variety of auxiliary means such as the use of physical properties, Patterson methods, introduction of heavy atoms, consideration of previously known structures, and the study of isomorphous or related compounds. In the special case of centrosymmetric structures the problem reduces to that of giving the positive or negative sign to the observed structure factor  $F_{hkl}$  for each of the observed  $hkl$  reflexions, possibly several hundred or more in number.

If a given arrangement of atoms is considered, it is possible to compute the value of  $F$  for all points in reciprocal space, *i.e.*, for a reflexion from a plane of any selected spacing and orientation. For centrosymmetric structures we may imagine the group of atoms arranged around the origin and repeated by simple translations of any desired lengths and directions to form a lattice. The resulting  $F$  plot in the reciprocal space is characteristic of the original arrangement and nature of the group of atoms. The value of  $F$  is seen to vary in magnitude from point to point and to undergo changes of sign. If a continuous variation of spacings could be made without other alterations in the structure, or at least with only such alterations as could be allowed for, it would be possible to observe a continuous variation in the magnitude of  $F$  and to determine the points where it vanished. These would correspond to changes of sign and hence all the signs could be found. An approximation to this procedure was adopted by Perutz<sup>17</sup> in a structure where the reflexions from a particular direction in a protein crystal are observable over a continuous range of spacings due to the taking up of variable amounts of liquid between layers. In ordinary practice the values of  $F$  observed from the Bragg reflexions are only those that correspond to the particular planes that occur in the crystal under investigation, *i.e.*, at certain comparatively widely separated points in reciprocal space and without the possibility of continuous variation. A. D. Booth,<sup>18</sup> however, has suggested a possible limited application of the method by the use of the diffuse reflexions. Although it is difficult owing to background to establish the existence of a point of zero intensity, it may be possible to show that there is no such point in a given region. Thus if a strong streak of diffuse X-ray scattering connects two regions in the reciprocal lattice of a centrosymmetric crystal the  $F$ 's corresponding to those two regions must have the same sign. This, it is suggested, might sometimes help to determine a few signs but would not go far towards solving the general problem. (Mrs.) K. Lonsdale<sup>19</sup>

<sup>17</sup> J. Boyes Watson and M. F. Perutz, *Nature*, 1946, **151**, 714.

<sup>18</sup> *Ibid.*, 1946, **158**, 380.

<sup>19</sup> *Ibid.*, p. 582.

points out that the argument is sound if the diffuse scattering is due to displacement or vibration of those atoms whose diffraction is mainly responsible for the reinforcing waves which give the Bragg reflexions, *i.e.*, the scattering in the streak and the two Bragg spots which it connects must be mainly due to the same atoms. In ice, however, where the contribution of the centrosymmetrically arranged oxygen atoms certainly decides the phases, strong diffuse streaks do connect regions where F's are *not* of the same sign. Moreover, the diffuse pattern is more symmetrical than could be the case if Booth's rule were satisfied. Any such attempts even at this very limited circumvention of sign computation must therefore be made with extreme caution.

*Inorganic Structures.—Crystal chemistry of neptunium and plutonium.*<sup>20</sup>

In the sexa-, quadri-, and ter-valent compounds the crystal radii decrease in the order uranium, neptunium, plutonium. The crystal chemistry of thorium and especially cerium is closely related to that of uranium, neptunium, and plutonium in the quadrivalent state. In the tervalent state the elements La—Sm show a marked similarity to uranium, neptunium, and plutonium in their crystal chemistry.

*Oxides, hydroxides, and basic salts.* The cell dimensions of a number of double oxides belonging to the perovskite type of structure have been accurately measured by H. D. Megaw.<sup>21</sup> This group of compounds includes structures of very varied but different symmetry, all based on small modifications of the same cubic cell. The ideal perovskite type includes  $\text{SrTiO}_3$ ,  $\text{SrSnO}_3$ ,  $\text{SrZrO}_3$ ,  $\text{BaSnO}_3$ ,  $\text{BaZrO}_3$ ,  $\text{BaThO}_3$ , and  $\text{BaTiO}_3$  above  $120^\circ$ . Some, including the usual form of  $\text{BaTiO}_3$ , have a tetragonal cell derived from the cubic structure by simple compression or extension along one of the fourfold symmetry axes. Others, including  $\text{CaTiO}_3$  (the mineral perovskite), are derived from the cubic structure by a shear in the 010 plane and a slight extension or compression along the  $b$  axis giving a monoclinic pseudo-cell with the  $a$  and  $c$  axes equal, so that the lattice is to be described as orthorhombic. Changes in some of the atomic parameters cause a doubling of the cell edges.  $\text{BaTiO}_3$  can also be prepared in a rhombohedral form. The pseudo-cell is obtained by a slight compression of the cubic cell along the cube diagonal but the true cell is a multiple of this. The occurrence of the various structure modifications is interpreted in a general way by steric considerations based on Goldschmidt's ionic radii.

Quenselite,<sup>22</sup>  $\text{PbMnO}_2(\text{OH})$ , has a structure characterised by the superposition of sheets of ions perpendicular to the  $a$  axis in the sequence Mn, O, Pb, OH, Pb, O, Mn. There is a good cleavage parallel to the sheets.

The decomposition products of lead dioxide at  $400^\circ$  in air have been investigated.<sup>23</sup> It was found that lead dioxide samples contained a small

<sup>20</sup> W. H. Zachariasen reporting at Institute of Physics Conference on War-time Progress in X-Ray Analysis, Royal Institution, July 1946; see also ref. (14).

<sup>21</sup> *Proc. Physical Soc.*, 1946, **58**, 133.

<sup>22</sup> A. Bystrom, *Arkiv Kemi, Min. Geol.*, 1945, **19**, A, 35.

<sup>23</sup> A. Westgren and H. Hägg, *ibid.*, 1945—1946, **20**, A, 11.

amount of water which probably forms part of the anion lattice as hydroxyl groups. The oxygen content cannot be below that corresponding to  $\text{PbO}_{1.95}$ . A decomposition product  $\alpha\text{-PbO}_x$  may also be obtained by oxidation of certain preparations of  $\text{PbO}$  in oxygen at  $300\text{--}350^\circ$ . This compound has a range of homogeneity with limits close to the formulæ  $\text{Pb}_3\text{O}_5$  and  $\text{Pb}_2\text{O}_3$ . The structure is very complicated and not determined with certainty. The next step in the decomposition is represented by  $\beta\text{-PbO}_x$ . The composition is near to  $\text{Pb}_2\text{O}_3$  and a structure is proposed in which the lead atoms occupy positions similar to those of the heavy atoms in cubic  $\text{Bi}_2\text{O}_3$  and cubic  $\text{Sb}_2\text{O}_3$ . It is concluded, contrary to the views of M. LeBlanc and E. Eberius,<sup>24</sup> that the tetragonal and orthorhombic modifications of  $\text{PbO}$  have no range of homogeneity or very narrow ones. Some preliminary data are also given which perhaps represent a third modification of  $\text{PbO}$ .

W. Feitknecht and W. Marti<sup>25</sup> have examined the products of oxidation of manganese(II) hydroxide and of ammoniacal manganese(II) salt solutions by oxygen and hydrogen peroxide. By powder photography the products are found to be  $\text{Mn(II),(III)}$  double hydroxide, hausmannite, hydrohausmannite,  $\alpha$ -,  $\beta$ -, and  $\gamma\text{-MnO(OH)}$ ,  $\text{Mn}_3\text{O}_4$ . Excepting hausmannite and  $\gamma\text{-MnO(OH)}$ , the degree of oxidation of the manganese in all these compounds can vary within certain limits, *i.e.*, they are non-Daltonian compounds. The manganites obtained from solutions of  $\text{Mn(II)}$  and other metals have a double layer structure with hexagonal layers of  $\text{MnO}_2$  and disordered hydroxide layers of the lower-valent metal such as  $\text{Ca,Mg,Zn}$ . The disorder is shown by varying sharpness of the inner rings or by their absence. W. Feitknecht<sup>26</sup> gives some data on basic cadmium sulphate, and W. Lotmar<sup>27</sup> has obtained single-crystal data from basic zinc chloride,  $\text{ZnCl}_2 \cdot 4\text{Zn(OH)}_2$ . This has a rhombohedral double layer structure, but no detailed parameter determination is made.

*Elements.* Precision measurements<sup>28</sup> on a sample of exceptionally pure lead, 99.999% by spectrographic analysis, give a unit cell dimension,  $4.9408 \pm 0.0001$  kX, slightly higher, as was to be expected, than previous values derived from samples that may have contained other atoms which are smaller than that of lead. Polonium<sup>29</sup> is shown to have two crystalline forms, a low-temperature structure described as simple cubic with  $a = 3.34$ , and a high-temperature simple rhombohedral form with  $a\ 3.36$  Å.,  $\alpha = 98^\circ 13'$ .

*Graphite.* The simple structure of graphite with its *ababab* sequence of layers was modified by Edwards and Lipson<sup>30</sup> on account of extra lines which indicate the presence of certain layers in the *abcabc* order, and J. Gibson<sup>31</sup> now reports the appearance of still further faint lines which are not accounted for by this arrangement. They have been observed in ordinary graphite and

<sup>24</sup> *Z. physikal. Chem.*, 1932, **A**, **160**, 69.

<sup>25</sup> *Helv. Chim. Acta*, 1945, **28**, 149.

<sup>26</sup> *Ibid.*, p. 1454.

<sup>27</sup> *Ibid.*, 1946, **29**, 14.

<sup>28</sup> H. P. Klug, *J. Amer. Chem. Soc.*, 1946, **68**, 1493.

<sup>29</sup> W. H. Beamer and C. R. Maxwell, *J. Chem. Physics*, 1946, **14**, 569.

<sup>30</sup> *Nature*, 1942, **149**, 328; *Ann. Reports*, 1942, **39**, 99.

<sup>31</sup> *Nature*, 1946, **158**, 752.

in very pure artificial graphites. Some of the lines are double, with an angular separation of  $0.20^\circ$ . No explanation has been given for these effects which might be due to other causes such as impure X-radiation.

"Amorphous" carbon. The term amorphous carbon has been used to describe more or less impure forms of carbon devoid of any obvious crystalline characteristics, but such materials prepared in a variety of ways all give essentially the same type of X-ray powder photograph with broadened diffraction maxima in the same positions. These have been interpreted as due to graphite-like structure with very small particles and varying disorder of the layers. It is now<sup>32</sup> found that a carbon prepared by carbonisation of hexaiodobenzene at  $5^\circ/\text{min.}$  up to  $1000^\circ$  in an atmosphere of nitrogen gives practically no coherent scattering of X-rays and thus seems to be almost completely without any ordered structure. Hexaiodobenzene was selected since the large iodine substituents might be expected to prevent a linking of two aromatic residues with their rings coplanar and so favour the formation of a hypothetical carbon structure in the form of a three-dimensional repetition of *o*-tetraphenylene residues. The material obtained is thought to consist of such a cross-linked structure, but highly disordered because of the presence of oxygen and hydrogen atoms, and it is suggested that small disordered chunks of this type of structure play some part in the building up of chars and cokes. Further evidence is required before this can be regarded as established.

*Ice and ammonium fluoride.* The diffuse X-ray scattering obtained from ice crystals gives further information on this structure.<sup>33</sup> The diffuse pattern is of thermal origin but cannot in the main be due to acoustical vibrations because no combination of elastic constants can give the star-shaped pattern found. Since the diffuse streaks cannot be due to oxygen (see above) there must be strong vibratory movement of the hydrogen nuclei. J. D. Bernal and R. H. Fowler<sup>34</sup> show that the unit cell must be at least three times as large as the apparent simple cell, while L. Pauling,<sup>35</sup> from considerations of the residual entropy, has concluded that the water molecules in ice cannot have definite orientations which would permit a unique crystalline configuration such as that of Bernal and Fowler. This new work by Lonsdale confirms Pauling's suggestion that change from one configuration to another is accomplished by group movements of hydrogen nuclei each of which would move from the neighbourhood of an oxygen atom to the next oxygen, or by rotation of water molecules. The small unit cell is therefore a statistical one, and even at low temperatures the apparent cell may be small owing to freezing in of different molecular configurations in different parts of the crystal. A similar star-shaped diffuse pattern is obtained for ammonium fluoride, isomorphous with ordinary ice. Some hailstones have been shown<sup>36</sup> to contain moderate sized single crystals of the ordinary ice form.

<sup>32</sup> J. Gibson, M. Holohan, and H. L. Riley, *J.*, 1946, 456.

<sup>33</sup> K. Lonsdale, ref. (19).

<sup>34</sup> *J. Chem. Physics*, 1933, **1**, 515.

<sup>35</sup> "Nature of the Chemical Bond", New York, 1939, p. 281.

<sup>36</sup> K. Lonsdale and P. G. Owston, *Nature*, 1946, **157**, 479.

*Methylammonium chloride.* The unit cell formerly ascribed to methylammonium chloride was based partly on powder-photograph measurements and was incorrect. The cell now<sup>37</sup> found contains two molecules. It is tetragonal, and the whole structure may be regarded as a somewhat distorted caesium chloride arrangement, in which methylammonium ions are surrounded by the chlorine ions of the top and bottom faces of the cell. The lengths of the methylammonium ions which point alternately up and down are parallel to the *c* axis, and from the Fourier analysis results the distance C—N =  $1.465 \pm 0.01$  Å. The predicted value is 1.47 if no allowance is made for the formal charge, but with such an allowance it is 1.44, so the formal charge appears not to have the expected effect although the differences here seem to be fairly close to the possible errors. In working out this structure it was found necessary to apply separate temperature factors for the methylammonium and the chlorine ions and an anisotropic temperature factor was used for chlorine with the maximum vibration along the *c* axis as is suggested by the form of the corresponding electron-density peak.

*Halogen-containing complexes.* Data concerning a structure determination of aluminium bromide<sup>38</sup> have now become available. Separate molecules of  $\text{Al}_2\text{Br}_6$  are arranged in a monoclinic cell to give a slightly deformed hexagonal close packing. The molecules are of the type found by Palmer and Elliot in the gaseous state by electron diffraction, *i.e.*, the molecules consist of two tetrahedra of bromine atoms around aluminium atoms, the tetrahedra sharing an edge. There are some marked differences in the intramolecular distances derived from the crystal structure and those given by electron diffraction. In particular the Al—Al distance 3.14 Å. is appreciably shorter than the 3.39 Å. found in the gaseous state, and in general the values found are closer to those that would be expected for a model constructed from two regular tetrahedra. The molecule is much less deformed than in the gaseous state, and it seems that the structure yields less to the repulsive force between the central aluminium atoms. A suggested explanation is that it would be impossible to obtain such a good packing with the more deformed molecules and there would be a consequent loss of van der Waals attraction between the bromine atoms.

The compound hitherto given the formula  $\text{Mo}_3\text{Cl}_4(\text{OH})_2 \cdot 8\text{H}_2\text{O}$  has been examined.<sup>39</sup> Analytical data suggest seven rather than eight water molecules, and although the unit-cell dimensions and density agree with six molecules of water, it is considered that seven is the more probable figure since the density determined may be low. From the structure determination the formula is now rewritten as  $[\text{Mo}_6\text{Cl}_8](\text{OH})_4 \cdot 14\text{H}_2\text{O}$ . The  $[\text{Mo}_6\text{Cl}_8]$  group is a slightly irregular cube with chlorine at each corner and with molybdenum atoms at the centres of each cube face but raised slightly, about 0.05 Å., above the faces. These groups are enclosed in a three-dimensional network of

<sup>37</sup> E. W. Hughes and W. N. Lipscombe, *J. Amer. Chem. Soc.*, 1946, **68**, 1970.

<sup>38</sup> P. A. Renes and C. H. MacGillavry, *Rec. Trav. chim.*, 1945, **64**, 276.

<sup>39</sup> C. Brosset, *Arkiv Kemi, Min. Geol.*, 1945—1946, **20** A 7.



oxygen atoms. Each molybdenum atom has four chlorine neighbours at 2.50, 2.57, or 2.62 Å. and one oxygen at 2.29 Å. In all, there are 18 oxygen atoms connected with one  $[\text{Mo}_6\text{Cl}_8]$  group. Of these, 4 must be OH and 14 must be water, although the 18 atoms are distributed in one group of 6 and one group of 12 equivalent point positions of the hexagonal cell. It is supposed that the 32 hydrogen atoms are distributed statistically among the 18 oxygen atoms. The oxygen-oxygen distances, which are not known with great certainty, are about 2.7 Å. The compound formerly described as  $[\text{Mo}_3\text{Cl}_4 \cdot 2\text{H}_2\text{O}]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$  has a tetragonal structure <sup>39a</sup> which contains the same  $\text{Mo}_6\text{Cl}_8$  group and is now rewritten  $[\text{Mo}_6\text{Cl}_8](\text{Cl}_4 \cdot 2\text{H}_2\text{O})$ . In the  $[\text{Mo}_6\text{Cl}_8]$  group the average Mo-Mo distance is 2.64 Å.

Precipitated potassium cryolite <sup>40</sup> has a variable composition depending on the fluorine-ion concentration at precipitation. The general formula is  $\text{K}_x\text{AlF}_{3+x}(\text{H}_2\text{O})_{3-x}$ ,  $x$  being between 2.9 and 3. With  $x = 2.9$  the compound is isomorphous with ammonium cryolite and has a cubic cell  $a = 8.41_8$  Å. The unit cell of  $\text{K}_3\text{AlF}_6$  is probably large and derived from a body-centred tetragonal cell  $a = 5.96$ ,  $c = 8.46_8$ . In precipitated potassium cryolite some  $[\text{AlF}_6]$  groups may be replaced by  $[\text{AlF}_5(\text{H}_2\text{O})]$  when the fluoride-ion concentration is not high enough. For every such group replaced one potassium ion is lost from the lattice.

Phosgenite,  $\text{Pb}_2\text{Cl}_2\text{CO}_3$ , and the isomorphous bromine compound have been examined.<sup>41</sup> A previous structure determination on phosgenite suggested that there were no carbonate groups in the structure but the arguments used are invalid since the unit cell determined now has the  $c$  dimension of the unit cell doubled. The intermediate reflexions that established this are exceptionally weak but are more pronounced in the corresponding bromide. The positions of lead and bromine have been determined and the rest of the structure inferred from packing considerations. It consists of lead, halogen, and carbonate ions. There is no evidence of linking to form Pb-Cl groups.

*Organic Crystals (General).*—The general constructional principles underlying the formation of organic crystals have been examined by W. Nowacki <sup>42</sup> who, in presenting the statistics for the compounds that have been suitably examined—a fraction of a per cent. only—points out that it remains to confirm the conclusions on the rest. However, the total number of compounds is considerable, about a thousand, and it seems unlikely that the high frequency of occurrence of certain space-groups which is familiar to workers in this field is accidental. G. Hägg <sup>43</sup> considered that the results might be influenced by the inclusion of a great number of space-group determinations which have been made on optically active substances, but Nowacki <sup>44</sup> replies with a table showing the frequencies before and after the subtraction of crystals which contain optically active molecules. In the first case in a total

<sup>39a</sup> *Arkiv Kemi, Min. Geol.*, 1946, **22**, A, 11.

<sup>41</sup> L. G. Sillen and R. Petterson, *ibid.*, p. 13.

<sup>43</sup> Quoted by Nowacki, ref. (42).

<sup>40</sup> *Ibid.*, 1946, **21**, A, 9.

<sup>42</sup> *Helv. Chim. Acta*, 1943, **26**, 459.

<sup>44</sup> *Helv. Chim. Acta*, 1945, **28**, 664.

of 914 compounds the group  $P2_1$  is found for 12%,  $P2_1/c$  for 10.5%, and  $P2_12_12_1$  for 22%. On elimination of 173 crystals with optically active molecules the statistics are not fundamentally altered the percentages being respectively 12, 7, and 22. Over 40% of the known organic structures therefore have these space-groups. Seven other space-groups, *viz.*,  $P1$  (2.4),  $C2$  (2.4),  $C2/c$  (3.4),  $P2_12_12$  (2.7),  $Pbca$  (2.1),  $Pnma$  (3.4) and  $C4/amm$ , account each for between 2 and 3.5% and leave about one-third of all the compounds for distribution among more than 200 remaining space-groups. In explanation of this, Nowacki says that the tendency to close packing, which is so frequently found in inorganic crystals, is certainly not a guiding principle, and quotes the 75% of all organic substances so far examined as having primitive lattices, with a further 16% having double primitive lattices, whereas a face-centred lattice, fourfold primitive, should lead to a maximum space filling. Although this is clearly so for the simplified case of cubic close packing of spheres, this part of the argument does not appear to the Reporter to be a strong one, since any structure may be referred, by a suitable choice of axes, to a primitive lattice, and in monoclinic crystals, for example, the investigator makes a deliberate choice of axes to avoid the selection of a cell centred in any way except, in some crystals, on (001) faces. Further, when the packing of awkward-shaped molecules is considered, it is found that by use of suitable symmetry operations the centres of molecules may be made to lie in positions closely approximating to those for a face-centred close packing although the structure as a whole is not formally centred, *e.g.*, in the structure of picryl iodide,<sup>45</sup> space-group  $P4_12_1$ .

In this connection also A. Kitaigorodsky,<sup>46</sup> by assuming intermolecular radii for each atom, C 1.70, H 1.18 Å., has calculated the proper volumes of a small number of aromatic hydrocarbons and compared them with the volumes per molecule in the crystal. Packing fractions between 0.68 and 0.72 are obtained and may be compared with the value 0.74 for closest packing.

For molecules which are markedly different in their extensions in different directions, centring which involves parallel repetition does not seem so effective for packing purposes as the use of symmetry operations which involve head-to-tail or similar packing. Apart from this, experience shows that molecules of the most diverse shapes tend to adopt arrangements in which the projecting portions of one fit into the indentations left by the surrounding molecules in such a way as to achieve a good degree of space filling. New structures often appear very striking not only in the manner whereby they maintain the familiar van der Waals separations of unlinked molecules, but also in the avoidance of any large gaps that would give inter-group separations appreciably greater than the normal. When open structures appear they are usually attributable to some special circumstance such as the directional requirements of hydrogen-bond linkages as in  $\alpha$ -resorcinol<sup>47</sup> or even more strikingly in quinol.<sup>48</sup> Nowacki further points out

<sup>45</sup> G. Huse and H. M. Powell, *J.*, 1940, 1398.

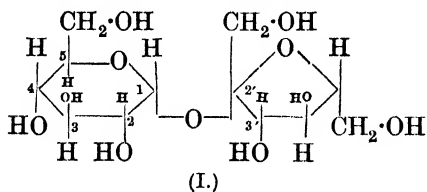
<sup>46</sup> *Acta Physicochim. U.R.S.S.*, 1946, 21, 379.

<sup>47</sup> J. M. Robertson, *Proc. Roy. Soc.*, 1936, A, 158, 79.

<sup>48</sup> Ref. (57).

that, since the majority of organic molecules have little symmetry of their own, any higher symmetry of the crystal must result from the arrangement of the molecules and thus selects the 92 asymmorphous space-groups as of special significance for organic crystals. Of all the compounds, 72% are found to belong to these space-groups. Since many of the molecules have an electric moment, the molecular arrangement will seek to bring about the most effective mutual saturation of dipoles. This is so when the molecules are arranged in zigzag chains. Only three symmetry elements achieve this, the two-fold screw axis  $2_1$ , a network of symmetry centres  $I$ , and a glide plane of symmetry  $c$ ,  $a$ ,  $b$ ,  $d$ , or  $n$ . On the assumptions that the favoured space-groups for organic structures obey the principles of (1) a primitive lattice, (2) asymmorphism, (3) symmetry elements permissible, are only those stated above either alone or in suitable combination, those to be expected are  $P2_1$ ,  $P2_1/c$ ,  $Pca$ ,  $Pna$ ,  $P2_12_1$  and  $Pbca$ . Some but not all of these occur in the list of commonly found space-groups, and a further limiting principle is introduced, that of efficient dipole saturation of one zigzag chain of molecules by the others. This requires that a two-fold screw axis may only be perpendicular to a glide plane, and thus leaves only  $P2_1$ ,  $P2_1/c$ , and  $P2_12_1$  as the specially preferred space-groups for organic crystals, *i.e.*, the three first mentioned as accounting for over 40% of the total. Among the other space-groups the number of examples is too small for any certain conclusions concerning their frequency, but some general tendencies can be understood; *e.g.*, in a comparable set of space-groups the frequency of occurrence increases with increase in the number of  $2_1$  screw axes as in  $P222$  (0.002%),  $P222_1$  (0.003),  $P2_12_12$  (2.7), and  $P2_12_12_1$  (10.4).

*Organic Structures.*—*Sucrose.* C. A. Beevers and W. Cochran<sup>49</sup> give a preliminary account of the structure of the sucrose molecule from an examination of the compound  $C_{12}H_{22}O_{11} \cdot NaBr \cdot 2H_2O$  and the isomorphous chloride. The heavy atoms simplify the phase-angle determinations. The



accepted structural formula of sucrose as 1- $\alpha$ -glucopyranose-2- $\beta$ -fructofuranose (I) is confirmed. Parameters for all atoms are given with an estimated error 0.5 Å. for interatomic distances and of 5° for bond angles. The oxygen

atoms attached to carbon atoms 1 and 2 are in the *cis*-configuration, and similarly those of 2' and 3'. The five atoms of the furanose ring are not coplanar, atoms 3', 4', 5' being displaced so as to bring the attached groups more nearly into the mean plane of the ring. Within the ring the mean C-C distance is given as 1.44 Å. and the mean angle as 104°. The pyranose ring is of the Sachse *trans*-(chair-shaped) form. This result should be compared with that obtained by E. G. Cox and G. A. Jeffrey<sup>50</sup> for glucosamine hydrobromide where the same form occurs, and by Cox, T. H.

<sup>49</sup> *Nature*, 1946, **157**, 872.

<sup>50</sup> *Ibid.*, 1939, **143**, 894.

Goodwin,<sup>51</sup> and A. I. Wagstaff who find the five carbon atoms in a plane with the oxygen atom out of the plane in methylated aldopyranoses. In the present compound each sodium ion is surrounded in a nearly regular octahedral manner by one bromine, two water molecules, and three hydroxyl groups, but the surroundings of the bromine are irregular.

*m*-Dinitrobenzene. An earlier attempted structure of *m*-dinitrobenzene led to a false conclusion through the deceptive character of the crystals which were assigned to a too high symmetry class. In a further examination of the structure<sup>52</sup> based on the space-group *Pbn* instead of *Pbnn* the molecule is found to be nearly planar. The results of the Fourier analysis are expressed in two diagrams (Fig. 1) projected on the plane of the benzene ring and at

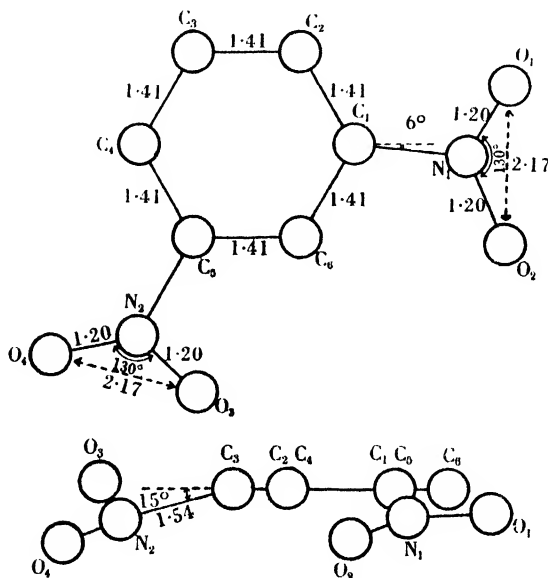


FIG. 1.

(Reproduced by permission from Proceedings of the Royal Society, 1946, A, 188, 59.)

right angles to it. This picture is, however, derived from one projection only, and the size and shape of the nitro-group were largely assumed from the results on other compounds. Some part of the small distortions from the symmetrical form of the molecule may be spurious. In the molecular compounds mentioned below the nitro-groups of 4 : 4'-dinitrodiphenyl have a mirror plane passing through the terminal carbon and the nitrogen atom perpendicular to the plane of the benzene rings but the carbon-nitrogen link is tilted slightly out of the plane of the ring. The determination of structures of aromatic nitro-compounds has been particularly beset with difficulties and there is scope for further accurate work.

**Molecular compounds.** Compounds of aromatic polynitro-compounds with

<sup>51</sup> *J.*, 1935, 1495.

<sup>52</sup> E. M. Archer, *Proc. Roy. Soc.*, 1946, A, 188, 51.

other aromatic substances frequently have a 1 : 1 ratio of the two molecules and this has sometimes been regarded as evidence for an electronic rearrangement which provides a chemical link of some kind between the components. It has also been suggested that the association of the two components might be explained in terms of various interactions (dipole induction effects, dispersion effect) between one molecule and the other without the necessity for a bond, and that these interactions are most effective if the planes of the aromatic rings are parallel.<sup>53</sup> Such a parallelism is observed in many crystalline molecular compounds of this type. W. S. Rapson, D. H. Saunder, and E. T. Stewart<sup>54</sup> have investigated the compounds of 4 : 4'-dinitrodiphenyl with various diphenyl derivatives and their results have a bearing on both these suppositions. Molecular complexes are formed only with 4-substituted and 4 : 4'-

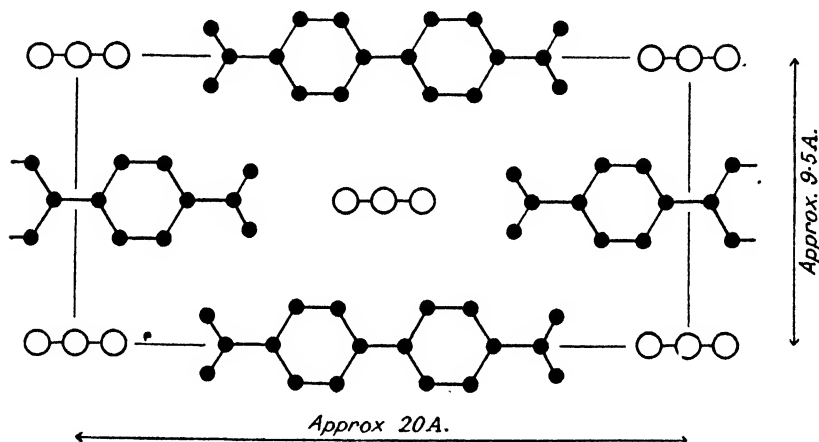


FIG. 2.

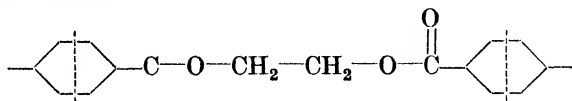
disubstituted diphenyls. The crystal structures of several of these have been examined and are all of the same general type, indicated in Fig. 2. In this idealised structure the dinitrodiphenyl molecules are arranged in planes one above the other separated by 3.7 Å. Running through the structure perpendicular to the planes of these molecules are channels in which the other component molecule, *e.g.*, 4-hydroxydiphenyl, is seen end on with its length perpendicular to the plane of the paper. None of the intermolecular distances is shorter than those normally found in crystals of aromatic nitro-compounds. These results therefore agree with those of H. M. Powell, G. Huse, and P. W. Cooke<sup>55</sup> on other compounds and reveal no localised bonding between the molecules. Diffuse X-ray reflexions and diffraction effects due to irregularities somewhat similar to those observed by G. Huse and H. M. Powell<sup>56</sup> in the compounds of hexamethylbenzene with picryl halides are observed. The molecular ratios in this new set of compounds are determined by geometrical considerations. They depend on the number

<sup>53</sup> D. H. Saunder, *Proc. Roy. Soc.*, 1946, A, 188, 21.<sup>55</sup> *Ibid.*, 1943, 153.<sup>54</sup> *J.*, 1946, 1110.<sup>56</sup> *Ibid.*, p. 435.

of dinitrodiphenyl layers that can be accommodated along the length of the other component molecule. Thus the length of the 4 : 4'-diacetoxy-diphenyl molecule, after allowance for approach of the next molecule in the end-on position, is 17—18 Å. This, divided by 3·7, the separation of the nitro-compound layers, gives  $n = 4\cdot6$ — $4\cdot9$  and the compound formed has a 5 : 1 ratio of the dinitro-compound to the other molecule. Similar agreement is found for the other molecules, the values of  $n$  being close to 4,  $3\frac{1}{2}$ , or 3 depending on the length of the molecule and in agreement with the compositions determined by analysis. These structures therefore show that neither the common 1 : 1 ratio of components nor parallelism of the aromatic rings is essential in these molecular compounds.

A preliminary communication by D. E. Palin and H. M. Powell<sup>57</sup> describes an entirely new type of relationship between the components of a molecular compound. Quinol forms a series of compounds of ideal formula  $3C_6H_4(OH)_2M$ , where  $M$  is a small molecule, *e.g.*, sulphur dioxide. The quinol molecules are linked through hydrogen bonds to form indefinitely extended cage structures in three dimensions. This structure, of a form imposed by the dimensions of the quinol molecules and the directional requirements of the hydrogen bonds, is of such an open character that a second identical framework structure can completely interpenetrate it. There is thus a mutual multiple enclosure of two giant molecules which have no direct linkages but are inseparable without the breaking of their own structures. This complex of interpenetrating molecules is still not very closely packed and contains cavities which are large enough to contain the small molecules which form the second component of the molecular compound. The formula is determined by the ratio of available cavities to the cage material, and  $M$  is restricted to such small molecules as will fit into the space. The enclosed material once trapped cannot escape despite the volatile nature of the component in the free state. Whether a given molecule  $M$  will form such a compound is determined, apart from size considerations, by the possibility of obtaining it in sufficient concentration in the same solution with quinol but does not otherwise depend on the chemical character of the second component.

*Fibres and other complex structures.* W. T. Astbury and C. J. Brown<sup>58</sup> report that terylene (polyethylene terephthalate) gives a well-oriented fibre diagram with spots that could be indexed on a triclinic unit cell. The fibre axis has the length of  $10\cdot8_8$  Å., which is compared with the  $10\cdot9$  Å. calculated for the repeat structure



Increasing disorientation is shown in the usual way by the drawing out of spots, but terylene is peculiar in that poorly oriented preparations give

<sup>57</sup> *Nature*, 1945, **156**, 335; see *J.*, 1947, 208.

<sup>58</sup> *Ibid.*, 1946, **158**, 871.

photographs like those of single crystals rotating about an axis inclined at a small angle to the principal axis. Spots are displaced to varying extents out of the layer lines, and an intense  $1\bar{1}0$  reflexion is seen as two overlapping spots one above and one below the equator. This means that in the drawing process it is more difficult to pull  $1\bar{1}0$  planes into parallelism. From the great intensity of this reflexion the chains must be approximately flat and parallel to  $1\bar{1}0$ . On drawing, chains or groups are first pulled straight by slipping parallel to this plane, and afterwards, with greater difficulty, these planes are themselves pulled into parallelism.

A new micro-method for X-ray diffraction of biological objects has been used by D. Kroger.<sup>59</sup> By its means a fibre pattern was obtained from a single starch grain. There were a considerable number of spots but the detailed structure has not been found. Diffraction patterns have previously been obtained with fairly simple small objects, such as a tungsten thread, and this extension seems to be of considerable importance.

Diffraction patterns of isoprene at  $20^\circ \text{K}$ . and  $80^\circ \text{K}$ . show many lines according to observations by C. J. B. Clews and A. Schallamach.<sup>60</sup> These establish the crystalline character of the material in these conditions but there is some difficulty in selecting a unit cell. Fibre patterns have been obtained with filaments of amylose and of amylose containing an uncertain, possibly variable amount of potassium hydroxide.<sup>61</sup>

The diffraction of X-rays by aqueous solutions of hexanolamine oleate<sup>62</sup> and of sodium oleate<sup>63</sup> has been studied, and a general structure for the monoöleyl disaturated triglycerides has been proposed.<sup>64</sup> The structure of soap micelles<sup>65</sup> has also been investigated. The X-ray diffraction effects in not too dilute aqueous solution indicate a structure of double layers of soap micelles with "water" layers between them. In the double layers the hydrocarbon chains are oriented towards each other with the polar ends towards the water. Micelle layer spacings are observed varying from 30 to 100 Å., and in the plane of the layers there is a nearly constant spacing of 4.5 Å. for normal paraffin-chain soaps at all concentrations from 4.5 to 30%. Addition of salts does not materially affect the short spacing, but potassium or sodium chloride produces a marked effect on micelle layer spacing and on the intensities of the X-ray pattern. The probable effect is that sodium chloride makes them smaller.

*Other structures.* A preliminary report<sup>66</sup> concerning zinc *p*-toluene-sulphonate and isomorphous substances of type  $(\text{CH}_3\cdot\text{C}_6\text{H}_4\cdot\text{SO}_3)_2\text{Zn}\cdot 6\text{H}_2\text{O}$  contains a Fourier electron-density projection which shows all atoms clearly with the exception of one of the oxygen atoms of the sulphonate group which overlaps with the sulphur atom. There is a regular octahedral arrangement

<sup>59</sup> *Nature*, 1946, **158**, 199.

<sup>60</sup> *Ibid.*, 1946, **157**, 160.

<sup>61</sup> F. R. Senti and L. P. Witnauer, *J. Amer. Chem. Soc.*, 1946, **68**, 2407.

<sup>62</sup> S. Ross and J. W. McBain, *ibid.*, p. 296.

<sup>63</sup> *Ibid.*, p. 547.

<sup>64</sup> L. J. Filer, S. S. Sidhur, B. F. Daubert, and H. E. Langenecker, *ibid.*, p. 167.

<sup>65</sup> W. D. Harkins, R. W. Mattoon, and M. L. Corrin, *ibid.*, p. 220.

<sup>66</sup> A. Hargreaves, *Nature*, 1946, **158**, 620.

of water molecules round each zinc atom. More precise details of the stereochemical relationships await a determination of the third atomic co-ordinate for each atom.

Unit cell dimensions have been given from two sources <sup>67</sup> for a number of diphenyltrichloroethane derivatives. One compound, *op'*-dichlorodiphenyltrichloroethane, has a triclinic cell with the unusual number of 20 molecules per unit cell. There must therefore be at least 10 molecules in the asymmetric unit, a state of things that may perhaps be attributed to the general awkwardness of the molecular shape for packing purposes. Wild and Brandenberger on the basis of Patterson analysis have suggested atomic positions for the chlorine atoms in DDT. Schneider and Fankuchen, who have also studied this substance, conclude that these suggested parameters require some modification, but details are not available. The highly symmetrical form of the quinuclidine molecule might lead one to suppose that it would form a hexagonal close packing, but this is not the case, since at room temperature it forms isotropic cubic crystals with  $a = 8.977 \pm 0.009$  Å. and four molecules per unit cell. The translation lattice is face-centred, *i.e.*, the molecule centres form a cubic close packing. In order to bring the trigonal symmetry of the molecule into agreement with the cubic symmetry there must be either free rotation of the molecules about their centres or a statistical disordered structure with the molecular trigonal axes parallel to the four sets of three fold axes of the cubic unit. On space considerations the latter is the more probable.

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<sup>67</sup> H. Wild and E. Brandenberger, *Helv. Chim. Acta*, 1946, **29**, 1024; M. Schneider and I. Fankuchen, *J. Amer. Chem. Soc.*, 1946, **68**, 2669.



# INORGANIC CHEMISTRY.

## 1. NON-STOICHEIOMETRIC COMPOUNDS.

RECENT work in inorganic chemistry has raised the question of the validity of the law of constant proportions, as applied to solid compounds. The existence and classification of "Berthollide" compounds have been noted previously in these Reports,<sup>1</sup> and it appears opportune to review our present state of knowledge of the subject.

It is generally recognised that the intermediate phases in metallic systems may exist over a range of composition, not necessarily including a rational chemical formula; an idealised chemical formula can usually be assigned, however, based on the composition of the unit cell of the crystal.<sup>2</sup> Between intermetallic and ionic compounds there is a transition, rather than an abrupt demarcation,<sup>3</sup> depending on the difference in electronegativity of the combining atoms, and whereas the elements of Groups VB, VIB, and VIIB form true salts with the most electropositive metals (Groups IA, IIA), their compounds with the transition and B-sub-group metals display a complete transition from the ionic to the quasi-metallic type. Variability of composition runs broadly parallel to sub-metallic properties, but is by no means limited to compounds of obviously sub-metallic character.

The distinction between solid solutions, interstitial compounds and non-stoichiometric compounds is, in the last analysis, rather arbitrary. N. S. Kurnakow<sup>4</sup> first proposed the term "Berthollide" (as distinct from "Daltonide") to describe homogeneous phases in systems where the maxima or minima of properties—melting point, conductivity, lattice order, etc.—do not coincide with a rational atomic ratio of the components. For the purpose of this report it is convenient to follow W. Schottky and C. Wagner<sup>5</sup> in considering the familiar "Daltonide" type as a special case of the "ordered mixed phase", a 2-component (or multicomponent) system with statistically regular lattice array.

Our present knowledge of crystal structure confers precise meaning on the term "solid solution" as applied to crystals of atomic lattice types. In a crystal phase of ideal formula  $AB_n$ , a stoichiometric excess of element B can be accommodated structurally in only three ways: (i) *Substitutional* solid solution: B atoms replace A atoms on lattice sites proper to A. (ii) *Interstitial* solid solution: additional B atoms are located in inter-lattice positions. (iii) *Subtractive* solid solution: all B atoms occupy proper B lattice sites, but a number of A lattice sites is left untenanted.

Since (ii) increases and (iii) decreases the average weight per unit cell, distinction between them is possible by combining density and X-ray cell

<sup>1</sup> *Ann. Reports*, 1933, **30**, 381; 1935, **32**, 211.

<sup>2</sup> A. Westgren, *Angew. Chem.*, 1932, **45**, 33.

<sup>3</sup> Cf. E. Zintl, *ibid.*, 1939, **52**, 1.

<sup>4</sup> *Z. anorg. Chem.*, 1914, **88**, 109.

<sup>5</sup> *Z. physikal. Chem.*, 1930, **B**, **11**, 163.

dimension measurements. In this way it was shown that in pyrrhotite,  $\text{FeS—FeS}_{1.14}$ ,<sup>6</sup> ferrous selenide,  $\text{FeSe—FeSe}_{1.15}$ ,<sup>7</sup> and wüstite,  $\text{FeO}_{1.06}$ — $\text{FeO}_{1.19}$ ,<sup>8</sup> the stoichiometric excess of non-metal represents a cation deficiency, the anion lattice being substantially complete. Thus a pyrrhotite  $\text{Fe}_3\text{S}_9$  is properly represented  $\text{Fe}_{0.89}\text{S}$ ; it cannot be regarded as a solid solution between two Daltonide compounds, and is a true non-stoichiometric compound. In the  $\epsilon$ -phase of the Fe—Sb system (ideally FeSb), increase in cell dimensions with increasing iron content above the ideal formula indicates that the excess of iron is accommodated interstitially.<sup>9</sup>

Substitutional solid solution is likely only in intermetallic compounds, where ionic repulsions would not be involved. Thus, in the  $\beta$ -phase of the Na—Pb system (27—35 atoms % Na; ideal composition,  $\text{NaPb}_3$ ), the stoichiometric phase lies outside the range of homogeneity; the stable phase has 4—9% of the Pb atoms replaced by Na.<sup>10</sup> According to M. J. Buerger,<sup>11</sup> in the marcasite-type  $\text{FeSb}_2$ ,  $\text{FeAs}_2$ ,  $\text{FeS}_2$ , the stoichiometric excess of iron usually present is substituted for a proportion of the non-metal. Such substitution in a metallic sulphide seems improbable, and this series could profitably be reinvestigated.

The *conditions of equilibrium* of lattice defects in a real crystal were worked out by W. Schottky and C. Wagner.<sup>4, 12</sup> In a stoichiometric crystalline compound MX, displacement of atoms from their regular lattice positions would be an endothermic process; the resulting interstitial atoms or vacant lattice sites could be distributed at random amongst any of the available positions of the crystal lattice. The defects therefore contribute substantially to the configurational entropy, as well as raising the total energy of the crystal, and it emerges that at all temperatures above 0° K. the free energy  $G (= H - TS)$  is a minimum for certain finite concentrations of lattice defects of each kind (depending on the energy involved in creating the defects). If interstitial M atoms, interstitial X atoms, vacant M sites, and vacant X sites are all present in significant concentrations, the equilibrium conditions are rather complex. However, if—as is reasonable for  $kT \ll$  energy of defect formation—it can be assumed that all types of defect are not equally probable, two simple limiting cases arise: (i) Equal concentrations of vacant cation sites and vacant anion sites (Schottky defects);<sup>13</sup> believed valid, e.g., for NaCl. (ii) Interstitial atoms of one or

<sup>6</sup> G. Hägg and G. Sücksdorf, *Z. physikal. Chem.*, 1933, B, **22**, 444; *Nature*, 1933, **131**, 167.

<sup>7</sup> G. Hägg and A. L. Kindstrom, *Z. physikal. Chem.*, 1933, B, **22**, 453.

<sup>8</sup> E. R. Jette and F. Foote, *J. Chem. Physics*, 1932, **1**, 29.

<sup>9</sup> A. Oftedal, *Z. physikal. Chem.*, 1927, **128**, 135; G. Hägg, *Z. Krist.*, 1928, **68**, 470.

<sup>10</sup> E. Zintl and A. Harder, *Z. physikal. Chem.*, 1931, A, **154**, 63.

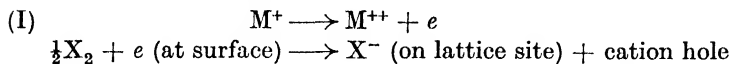
<sup>11</sup> *Amer. Min.*, 1934, **19**, 37.

<sup>12</sup> W. Schottky, *Z. physikal. Chem.*, 1935, B, **29**, 335; R. H. Fowler and E. A. Guggenheim, "Statistical Thermodynamics", Cambridge, 1939, paras. 1302, 1303.

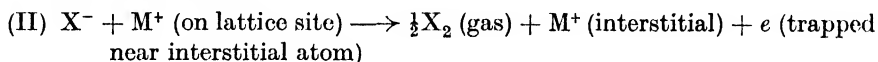
<sup>13</sup> W. Schottky, *Naturwiss.*, 1935, **23**, 656; *Z. physikal. Chem.*, 1935, B, **29**, 335.

other kind, with a corresponding number of vacant lattice sites (Frenkel defects); <sup>14</sup> example, AgBr.

The stoichiometric compound is, however, a limiting case. For the crystal in contact with the vapour of one of its components (*e.g.*, a diatomic non-metal, such as O<sub>2</sub>, I<sub>2</sub>, etc.), we must consider the possible addition or removal of X<sup>-</sup> ions at the surface of the originally stoichiometric crystal, by such processes as (I) or (II).



*Addition* of supernumerary X<sup>-</sup> ions to the crystal involves (a) an increase in valency of a corresponding number of M<sup>+</sup> ions and (b) the creation of vacant cation sites which will ultimately distribute themselves by diffusion throughout the lattice.



*Removal* of X<sup>-</sup> ions from the lattice involves (a) effective conversion of the same number of M<sup>+</sup> cations into M atoms and (b) creation of interstitial atoms or (for crystals with Schottky defects) vacant anion sites.

Addition or removal of X ions will accordingly involve changes both in total energy and in configurational entropy as compared with the stoichiometric crystal. The minimum free energy for any temperature and any given pressure  $p_x$  of the vapour X<sub>2</sub> corresponds (for a crystal with Frenkel defects) with concentrations of defects given \* by

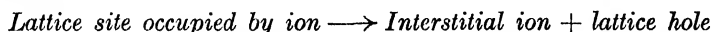
$$N^h = N^l \cdot p_x^{\frac{1}{2}} \cdot \exp. - E_x/kT \quad . \quad . \quad . \quad (1)$$

$$N^i = N^l \cdot p_x^{-\frac{1}{2}} \cdot \exp. - (E^h + E^i + E_x)/kT \quad . \quad . \quad . \quad (2)$$

where  $N^h$ ,  $N^i$  = number of M holes and interstitial M atoms in a crystal containing  $N^l$  cations;  $E^h$ ,  $E^i$  = energy expenditure to produce one vacant M site or interstitial M atom in the stoichiometric crystal,  $E_x$  = expenditure of energy in adding one additional X atom to the crystal. The concentrations of holes and interstitial atoms are not independent, being related by

$$\frac{N^i \cdot N^h}{(N^l)^2} = \exp. - (E^h + E^i)/kT \quad . \quad . \quad . \quad (3)$$

(3) defines in effect the equilibrium constant of a quasi-chemical dissociation :



For the stoichiometric crystal,

$$N^h*/N^l = N^i*/N^l = \delta = \exp. -(E^h + E^i)/2kT \quad . \quad . \quad (4)$$

$\delta$  is the intrinsic disorder of the stoichiometric crystal, which is in equilibrium with one particular partial pressure of the component X only.

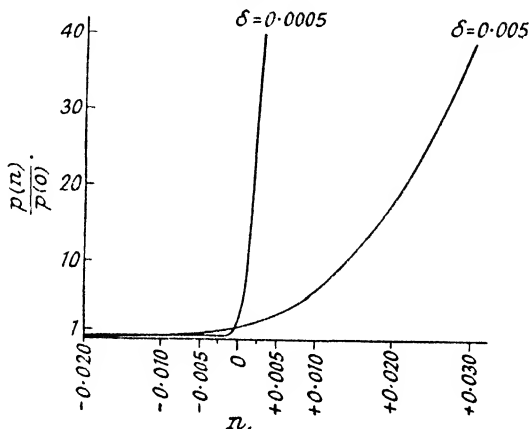
<sup>14</sup> J. Frenkel, *Z. Physik*, 1926, **35**, 652.

\* Approximately, the contributions to the vibrational energy being neglected (*cf.* Mott and Gurney, "Electronic Processes in Ionic Crystals", Oxford, 1940, p. 29).

For any other pressure of X the crystal will contain at equilibrium a stoichiometric excess (or deficiency) of X given by  $N^h - N^i$ , which depends upon the intrinsic disorder of the stoichiometric phase. Writing  $(N^h - N^i)/N^i = n$ , and the pressures of X in equilibrium with  $\text{MX}_{1.000}$ ,  $\text{MX}_{1+n}$  as  $p(0)$ ,  $p(n)$ , respectively, we have

$$\frac{p(n)}{p(0)} = \frac{n}{2\delta} + \left\{ \left( \frac{n}{2\delta} \right)^2 + 1 \right\}^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad . \quad (5)$$

Variation of stoichiometric defect for two values of  $\delta$  is illustrated in the figure. If the stoichiometric crystal is almost perfectly ordered, the



equilibrium pressure must increase very steeply to produce even small changes in composition.

The essential consequences of this thermodynamic theorem are as follows.

(i) It is perfectly general, suggesting potential variability of composition for all ionic, semimetallic, or intermetallic compounds.

(ii) Unless the degree of lattice disorder in the stoichiometric compound is appreciable, variation of composition under experimentally accessible equilibrium conditions may be imperceptibly small. The "Daltonide" compound thus appears as a special case.<sup>15</sup>

(iii) The intrinsic disorder  $\delta$  will be small unless production of defects is not too endothermic, as compared with the thermal energy.  $E^h$ ,  $E^i$  are smaller than the lattice energy of the crystal by a factor depending<sup>16</sup> on the polarisation and distortion of the crystal lattice around each defect. Non-ionic interactions (e.g., van der Waals forces) between the atoms are particularly important in stabilising defects and in favouring the location of atoms or ions in interstitial positions. Compounds of the transition or

<sup>15</sup> A. Ölander, *Z. physikal. Chem.*, 1933, A, **165**, 65.

<sup>16</sup> W. Jost, *J. Chem. Physics*, 1932, **1**, 466; W. Jost and G. Nehlep, *Z. physikal. Chem.*, 1936, B, **32**, 1; N. F. Mott and M. J. Littleton, *Trans. Faraday Soc.*, 1938, **34**, 485; W. Jost, *ibid.*, 1938, **34**, 860.

B-sub-group metals are therefore likely to possess a higher degree of intrinsic disorder than are structures built up from the inert-gas-like ions. Estimates of  $\delta$  in typical compounds have been made by J. Addink<sup>17</sup> and by E. Koch and C. Wagner.<sup>18</sup>

(iv) Deviation from stoichiometry involves a valence change. *Excess metal* may be incorporated by converting some cations effectively into neutral atoms, or into cations of lower valency. This decrease in valency is possible for any cation, including the inert-gas-like cations. The alkali halides, heated in the vapour of the corresponding metal, will take up a few atoms per thousand of excess metal.<sup>19</sup> *Excess non-metal* involves the presence either of cations of higher valency (energetically permissible only in compounds of the metals displaying variable valency), or of anions of lower valency. The low binding energy renders the latter source of stoichiometric variation less favoured, but potassium iodide (*e.g.*) will incorporate a stoichiometric excess of iodine (up to a few atoms per million  $I^-$  ions).<sup>19, 20</sup> It seems likely that supernumerary  $S_2$  molecules may be built into the pyrite structure on lattice sites proper to  $S_2^{2-}$  groups (*vide infra*,  $NiS_2$ ,  $CoS_2$ ).

For a compound to be stable over an appreciable range of composition, certain conditions must evidently be fulfilled.<sup>21</sup> The energy expenditure to produce defects must not be too large; the energy difference between the two valency states involved must also be small; the difference in size between the ions in the two valency states must be small, so that the lattice may not be distorted to the point of collapse. In all these respects the compounds of the heavier metals occupy a special position, and it is amongst these that marked variations from stoichiometric simplicity have been encountered.

The Schottky-Wagner theory makes no reference to factors limiting the range of existence of a crystal phase, and is strictly valid only where the concentration of lattice defects is very small. The tolerance of a crystal lattice for excess of its components is actually limited. The positions of all atoms adjacent to an interstitial atom, lattice hole or ion of higher valency must undergo adjustment, and if the concentration of such lattice disturbances exceeds some limiting value, the crystal lattice may break up to give a second phase and a structure "saturated" with defects. An attempt to include this within the scope of the theory has been made<sup>22</sup> by considering not only the energy expenditure to produce lattice defects, but also an energy of interaction of defects when adjacent to each other. The effect of this is that the distribution of defects through the crystal lattice is no longer completely random. Below a critical temperature

<sup>17</sup> *Nature*, 1946, **157**, 764. These estimates include, and are probably dominated by, the effects of secondary structure,  $\delta$  being much smaller still.

<sup>18</sup> *Z. physikal. Chem.*, 1937, **B**, **38**, 295.

<sup>19</sup> Cf. R. W. Pohl, *Proc. Physical Soc.*, 1937, **39**, Extra part, 1.

<sup>20</sup> E. Mollwo, *Ann. Physik*, 1937, **29**, 304.

<sup>21</sup> W. Klemm, *Atti X Cong. int. Chim.*, Rome, 1938, **2**, 696; *Die Chemie*, 1943, **56**, 6.

<sup>22</sup> J. S. Anderson, *Proc. Roy. Soc.*, 1946, **A**, **185**, 69.

(dependent on the interaction energy) the lattice is stable only when the concentration of defects is less than a limiting value; if this is exceeded, the non-stoichiometric phase breaks up into a 2-phase system. As the saturation concentration of (*e.g.*) vacant cation holes and interstitial cations will in general be different, the range of accessible compositions on the metal-poor and the metal-rich side of the ideal formula can differ widely. In particular, the maximum permitted concentration of interstitial atoms could be less than would correspond to the intrinsic disorder  $\delta$  of the lattice of the stoichiometric compound. The ideal composition would then fall within the 2-phase region, and the stoichiometric compound would be unstable: only the phase with a stoichiometric excess of non-metal would exist. In a number of well-established instances (see below) this is found to be the case. This model is over simplified but reproduces some typical features of equilibria involving non-stoichiometric phases.

*Occurrence of Non-stoichiometry in Binary Compounds.*—Apart from some compounds of variable composition long known from their mineral occurrence (*e.g.*, pyrrhotite), evidence for the existence of non-stoichiometric compounds has come principally from studies of phase equilibria in binary systems, and needs critical examination. In metallic and quasi-metallic systems, the standard methods of thermal analysis may reveal the range of stability of intermediate phases. Where one component is volatile (*e.g.*, in oxide and sulphide systems), study of the  $(p, X)_T$  equilibrium is convenient, as used in the long series of memoirs from the Göttingen and Hanover schools of W. Biltz.<sup>23</sup> If a solid phase has a range of composition, the system is bivariant over the same range; the equilibrium pressure varies with the composition of the solid phase instead of changing abruptly from one univariant equilibrium to another at the composition of each stoichiometric solid compound. However,  $(p, X)$  isotherms or  $(T, X)$  isobars of similar shape can result from a completely different cause: formation of the product of a reaction in a stoichiometric but "active" state—*e.g.*, imperfectly crystallised or having high surface energy owing to its state of subdivision<sup>24</sup>—whereby false equilibria are set up. This has too frequently been overlooked, and conclusions drawn from the shape of degradation curves have in several instances (*cf.* lead and antimony oxides, below) subsequently been found incorrect. Where, as in such systems as  $\text{NiS-NiS}_2$ <sup>25</sup> and  $\text{CoS-CoS}_2$ <sup>26</sup> the experimental measurements have been made at temperatures high enough for recrystallisation and true equilibration (diffusion is appreciable above  $0.5 \times$  absolute melting point),<sup>27</sup> the

<sup>23</sup> Key papers to the experimental method are: W. Biltz and H. Müller, *Z. anorg. Chem.*, 1927, **163**, 257; W. Biltz and R. Juza, *ibid.*, 1930, **190**, 162; F. Wiechmann, M. Heimbürg, and W. Biltz, *ibid.*, 1939, **240**, 129.

<sup>24</sup> R. Fricke, *Naturwiss.*, 1943, **31**, 469; G. F. Hüttig and F. Kölbl, *Z. anorg. Chem.*, 1933, **214**, 289.

<sup>25</sup> W. Biltz, A. Voigt, K. Meisel, F. Weibke, and P. Ehrlich, *ibid.*, 1936, **228**, 273.

<sup>26</sup> O. Hülsmann and W. Biltz, *ibid.*, 1935, **224**, 73.

<sup>27</sup> G. Tammann, *ibid.*, 1925, **149**, 67.

evidence of ( $p, X$ ) isotherms is significant. Less weight attaches to evidence obtained similarly for oxide systems. The melting points of oxides are usually so high that "active" states hamper the attainment of real equilibria.

X-Ray studies have been widely used alone or in conjunction with ( $p, X$ )<sub>T</sub> or ( $T, X$ )<sub>p</sub> measurements. Where the diffraction lines of the minimum detectable amount of a new phase must be sought, the tendency is to exaggerate the range of existence of a phase. A narrow, but finite, range of existence may equally well be overlooked. Most conclusive, but used hitherto in too few instances, is the precise measurement of cell dimensions. Occurrence of a non-stoichiometric phase can generally be unequivocally detected, and the mode of incorporation of the excess of one component can be deduced.<sup>5, 28, 29, 30</sup> With paramagnetic or ferromagnetic compounds of the transition metals, magnetic measurements can be used to determine the phase boundaries.<sup>31, 32</sup>

Minute departures from stoichiometric balance, below any limit of analytical detection, may still be detected through the electronic semi-conducting properties they confer on otherwise non-conducting crystals.<sup>33</sup> Each atom of excess metal represents a supernumerary cation + trapped electron in the lattice, and constitutes a filled impurity level from which, by the fluctuations of thermal energy, the electron may be excited to the conduction band of the crystal. A cation of higher valency, in a crystal with excess non-metal, is a site of electron deficiency, an empty impurity level to which an electron may be excited from the originally filled valency band of the crystal.<sup>34</sup> Electrons in the first case, or "positive holes" in the second case, are thereby rendered mobile, giving rise to electronic conductivity but differing in respect of the sign of certain consequential effects (Hall effect, thermoelectric effect).<sup>35</sup> The relation of semiconducting properties to variability of composition is revealed, for metallic oxides, by the effect of the oxygen pressure. Diminution of oxygen pressure *increases* the conductivity of oxides derived from the highest valency state of a metal (stoichiometric excess of metal increased), and *decreases* the (positive hole) conductivity of oxides derived from lower valency states (stoichiometric excess of oxygen decreased).<sup>36</sup> Even the most refractory oxides, like Al<sub>2</sub>O<sub>3</sub> and CaO, become metal-excess conductors at high temperatures; <sup>37</sup> colour changes such as those of ZnO, In<sub>2</sub>O<sub>3</sub>, CeO<sub>2</sub> are associated with the reversible loss of oxygen atoms from the crystal lattice. C. Wag-

<sup>28</sup> G. Hägg and G. Söderholm, *Z. physikal. Chem.*, 1935, B, **29**, 88.

<sup>29</sup> H. Haraldsen, *Z. anorg. Chem.*, 1937, **234**, 372.

<sup>30</sup> W. Klemm and N. Fratini, *ibid.*, 1943, **251**, 222.

<sup>31</sup> H. Haraldsen, *ibid.*, 1937, **231**, 78; 1941, **246**, 169, 195.

<sup>32</sup> H. Haraldsen and F. Mehmed, *ibid.*, 1938, **239**, 369.

<sup>33</sup> C. Wagner, *Z. physikal. Chem.*, 1933, B, **22**, 181.

<sup>34</sup> For a general review, cf. F. Seitz, *J. Appl. Physics*, 1945, **16**, 553.

<sup>35</sup> For a general review, cf. R. J. Maurer, *ibid.*, p. 563.

<sup>36</sup> E. Friederich, *Z. Physik*, 1925, **31**, 813; W. Meyer, *ibid.*, 1933, **85**, 278.

<sup>37</sup> W. Hartmann, *ibid.*, 1936, **102**, 709.

ner<sup>38</sup> has sought to follow the ( $p, X$ ) equilibria in the ZnO and the Cu<sub>2</sub>O systems on the basis of plausible assumptions as to the relation between conductivity and stoichiometric excess, but his assumptions are in doubtful accord with the whole range of experimental facts.<sup>39</sup>

The following survey is not exhaustive, and includes only those compounds for which explicit evidence has been cited; intermetallic compounds are omitted. Non-stoichiometric phases are indicated (cf. Klemm)<sup>21</sup> by a bar above the idealised formula.

(I) *Hydrides*.—The interstitial semimetallic hydrides of Zr, Th, Ta and the rare-earth metals approach stoichiometric compositions only as an upper limit of hydrogen content.<sup>40</sup> The essentially Berthollide Pd-H equilibria were discussed from the statistical thermodynamic viewpoint by J. R. Lacher,<sup>41</sup> and it has recently been shown<sup>42</sup> that the Zr-H system has similar characteristics when the complicating effect of oxygen, present in interstitial solution in the metal—which vitiated much of Sieverts's work—is avoided. The complete equilibria might be interpreted along the lines indicated in ref. (22).<sup>43</sup>

(II) *Sulphides, Selenides, Tellurides*.—The table collects data for MX and MX<sub>2</sub> compounds of the first transition series, but omits the (quasi-metallic) subsulphides, etc., some of which (*e.g.*, pentlandite Ni<sub>9</sub>S<sub>8</sub><sup>44</sup> and the Co<sub>4</sub>S<sub>3</sub> phase<sup>45</sup>) undoubtedly have a range of existence. In every case, the MX compounds with NiAs type structure exist over a wide range of composition (contrast MnS and the low-temperature forms of FeSe and NiS) through the omission of cations from the structure. In some cases at least (*e.g.*, CoS) the stoichiometric compound is unstable. In the light of the theoretical discussion (equation 5) it is important that the proportion  $\delta$  of vacant sites of both kinds in the stoichiometric phase may apparently be as high as 5–7%.<sup>49</sup> The complete transition between VSe and VSe<sub>2</sub>, CoTe and CoTe<sub>2</sub>, NiTe and NiTe<sub>2</sub> is particularly noteworthy. The NiAs and the CdI<sub>2</sub> structures are so related that the former is transformed into the latter by the ordered omission of half the cations. Thus, in the V-Se system,

<sup>38</sup> H. H. von Baumbach and C. Wagner, *Z. physikal. Chem.*, 1933, **B**, **22**, 199; H. Dunwald and C. Wagner, *ibid.*, p. 212; J. Gundermann, K. Hauße, and C. Wagner, *ibid.*, 1937, **B**, **37**, 148.

<sup>39</sup> Cf. B. Gudden, *Ergebn. exakt. Naturwiss.*, 1934, **13**, 222; J. S. Anderson and M. C. Morton, *Proc. Roy. Soc.*, 1945, **A**, **184**, 83; *Trans. Faraday Soc.*, in the press.

<sup>40</sup> A. Sieverts *et al.*, *Z. anorg. Chem.*, 1926, **153**, 289; 1930, **187**, 155; 1928, **172**, 1; 1931, **199**, 384.

<sup>41</sup> *Proc. Roy. Soc.*, 1937, **A**, **161**, 525.

<sup>42</sup> M. N. A. Hall, S. L. H. Martin, and A. L. G. Rees, *Trans. Faraday Soc.*, 1945, **41**, 306.

<sup>43</sup> Dr. A. L. G. Rees, private communication.

<sup>44</sup> Cf. J. E. Hawley, G. L. Colgrove, and H. F. Zurbrigg, *Econ. Geol.*, 1943, **38**, 335.

<sup>45</sup> O. Hülsmann and F. Weibke, *Z. anorg. Chem.*, 1936, **227**, 113.

<sup>46</sup> W. Biltz, P. Ehrlich, and K. Meisel, *ibid.*, 1937, **234**, 97.

<sup>47</sup> W. Klemm and E. Hoschek, *Z. anorg. Chem.*, 1939, **242**, 49.

<sup>48</sup> W. Biltz and A. Köcher, *ibid.*, 1939, **241**, 324.

<sup>49</sup> H. Haraldsen, *ibid.*, 1937, **234**, 372; H. Haraldsen and A. Neuber, *ibid.*, p. 337.



*Compounds MX<sub>n</sub> of Transition Metals.*

	Com- pound.	Struc- ture.	<i>n</i> .	Ref.	Com- pound.	Struc- ture.	<i>n</i> .	Ref.	Com- pound.	Struc- ture.	<i>n</i> .	Ref.
Ti	$\overline{\text{TiS}}$		1.0—1.1	46								
	$\overline{\text{TiS}}_{1.5}$		1.1—1.5									
	$\overline{\text{TiS}}_2$	<i>C6</i>	1.5—2.0 *									
V	$\overline{\text{VS}}$	( <i>a</i> ) <i>B8</i>	1.0—1.16	47	$\overline{\text{VSe}}$	( <i>a</i> ) <i>B8</i>	0.98—1.2	47				
	$\overline{\text{VS}}_{1.5}$	( <i>β</i> ) <i>M</i>	1.17—1.53	48	$\overline{\text{VSe}}_{1.5}$	( <i>β</i> ) <i>M</i>	1.2—1.6					
					$\overline{\text{VSe}}_2$	( <i>γ</i> ) <i>C6</i>	1.6—2.0					
Cr	$\overline{\text{CrS}}$	( <i>a</i> ) <i>B8</i>	1.0—1.17	49	$\overline{\text{CrSe}}$	( <i>a</i> ) <i>B8</i>	1.0—1.15	32	$\overline{\text{CrTe}}$	( <i>a</i> ) <i>B8</i>	<1.0—1.17	50
	$\overline{\text{CrS}}_{1.5}$	( <i>β</i> ) <i>M</i>	1.22—1.48		$\overline{\text{CrSe}}_{1.33}$	( <i>β</i> ) <i>M</i>	1.20—1.33		$\overline{\text{CrTe}}_{1.3}$	( <i>β</i> , <i>γ</i> )	1.2—>1.5	
					$\overline{\text{CrSe}}_{1.5}$	( <i>γ</i> ) <i>H</i>	1.44—1.50					
Mn	$\overline{\text{MnS}}$	( <i>a</i> ) <i>B1</i>	1.00	51								
	$\overline{\text{MnS}}_2$	( <i>β</i> ) <i>B3</i>										
		<i>C2</i>	2.00 .									
Fe	$\overline{\text{FeS}}$	<i>B8</i>	1.0—1.14	52	$\overline{\text{FeSe}}$	<i>T</i>	1.00	6	$\overline{\text{FeTe}}$	<i>B8</i>	<1.0—?	55
				53	$\overline{\text{FeSe}}$	( <i>a</i> ) <i>B8</i>	1.0—1.13					
				54		( <i>β</i> ) <i>M</i>	1.13—1.31			<i>C18</i>	?	
	$\overline{\text{FeS}}_2$	<i>C2</i> , <i>C18</i>	1.95—2.05	5	$\overline{\text{FeSe}}_2$	<i>C18</i>	?					
Co	$\overline{\text{CoS}}$	<i>B8</i>	1.05—1.25	26					$\overline{\text{CoTe}}$	<i>B8</i>		
	$\overline{\text{CoS}}_{1.33}$	<i>H11</i>	1.333	56					$\overline{\text{CoTe}}_2$	{ <i>C6</i> <i>C18</i> }	1.0—2.0	57
	$\overline{\text{CoS}}_2$	<i>C2</i>	1.9—>2.0	45	$\overline{\text{CoSe}}_2$	<i>C2</i>	?	57			?	
Ni	$\overline{\text{NiS}}$	<i>B13</i>	1.00	58								
	$\overline{\text{NiS}}$	<i>B8</i>	1.0—1.2						$\overline{\text{NiTe}}$	<i>B8</i>	1.0—2.0	57
	$\overline{\text{NiS}}_2$	<i>C2</i>	2—>3		$\overline{\text{NiSe}}_2$	<i>C2</i>	?	57	$\overline{\text{NiTe}}_2$	<i>C6</i>		59

\* At high temperatures; range of existence narrower at low temperatures.

*B1*, NaCl type. *B3*, ZnS type. *B8*, NiAs type. *B13*, trigonal millerite type. *C6*, CdI<sub>2</sub> type. *C2*, pyrite type. *C18*, marcasite type. *H11*, spinel type. *M*, *H*, *T*, monoclinic, hexagonal, and tetragonal phases of other or unknown structural types.

the  $\alpha$ -phase is stable with up to 17% of the cation sites vacant, the "holes" being distributed at random. A corresponding proportion of  $V^{2+}$  ions is replaced by  $V^{3+}$  or  $V^{4+}$ . Further increase in the concentration of cation holes initiates an ordering process which lowers the crystal symmetry ( $\beta$ -phase). Finally, with 38–50% of the original cation sites vacant, the holes are segregated largely into alternate cation sheets of the original structure. Another hexagonal,  $CdI_2$ -type structure results, ideally  $VSe_2$ , but including up to 20% extra cations (partial replacement of  $V^{4+}$  by  $V^{2+}$ ). Only in exceptionally favourable cases can the range of existence be as wide as this, and the gaps of miscibility as narrow, but a similar sequence of changes is met with in some oxide systems.

$\overline{Cu_2S}$ ,  $\overline{Cu_2Se}$ ,  $\overline{Cu_2Te}$ . The high-temperature modifications of these, with a random distribution of cations,<sup>60</sup> are stable with a wide range of cation deficiency; certainly up to  $Cu_{1.7}S$ ,  $Cu_{1.6}Se$ ,  $Cu_{1.65}Te$ . Certain properties—conductivity, self-diffusion, etc.—have a maximum value close to the composition  $Cu_{1.8}X$ .<sup>61, 62</sup> The  $\overline{Cu_2S}$  phase is of considerable mineralogical interest; N. W. Buerger<sup>63</sup> considers that cubic chalcocite has the ideal composition  $Cu_{1.8}S$  (i.e., 10% of cations missing) and is distinct from the  $\overline{Cu_2S}$  phase proper.  $CuS$  does not appear to have a measurable range of existence.<sup>64</sup> As befits the instability of higher valency states of silver,  $Ag_2S$  is not stable over any wide range of composition, but does take up a measurable excess of sulphur.<sup>65</sup> Equilibrium compositions at 300° are

Vapour pressure of S, mm. ....	0	0.6	5.2	21
Composition.....	$Ag_{2.000}S$	$Ag_{1.9922}S$	$Ag_{1.997}S$	$Ag_{1.998}S$

(III) *Arsenides, etc.*—Transition metals form compounds  $MX$ ,  $MX_2$ , with  $NiAs$  and (mostly) marcasite structures respectively; few systems have been investigated thoroughly. Löllingite,  $FeAs_2$ , usually contains an

<sup>60</sup> H. Haraldsen and A. Neuber, *Z. anorg. Chem.*, **234**, 353.

<sup>61</sup> W. Biltz and F. Wiechmann, *ibid.*, 1936, **228**, 268.

<sup>62</sup> R. Juza and W. Biltz, *ibid.*, 1932, **205**, 275; H. S. Roberts, *J. Amer. Chem. Soc.*, 1935, **57**, 1034; H. Haraldsen, *Z. anorg. Chem.*, 1937, **231**, 78; 1941, **246**, 169, 195; *Z. Elektrochem.*, 1939, **45**, 370; E. Jensen, *Amer. J. Sci.*, 1942, **240**, 695; J. J. Lukes, C. F. Prutton, and D. Turnbull, *J. Amer. Chem. Soc.*, 1945, **67**, 697.

<sup>63</sup> Ref. (11).

<sup>64</sup> F. G. Smith, *Amer. Min.*, 1942, **27**, 1.

<sup>65</sup> A. Oftedal, *Z. physikal. Chem.*, 1928, **132**, 208.

<sup>66</sup> H. Haraldsen, *Z. anorg. Chem.*, 1935, **224**, 85; M. Heimbrecht, W. Biltz, and K. Meisel, *ibid.*, 1939, **242**, 229.

<sup>67</sup> S. Tengnér, *ibid.*, 1938, **239**, 127.

<sup>68</sup> Ref. (25).

<sup>69</sup> Ref. (30).

<sup>70</sup> P. Rahlfs, *Z. physikal. Chem.*, 1936, *B*, **31**, 157.

<sup>71</sup> H. Reinhold and H. Möhring, *ibid.*, 1937, *B*, **38**, 221; H. Reinhold and H. Seidel, *ibid.*, p. 245.

<sup>72</sup> H. Reinhold and H. Brauninger, *ibid.*, 1938, *B*, **41**, 397.

<sup>73</sup> *J. Chem. Physics*, 1939, **7**, 1067; *Econ. Geol.*, 1941, **36**, 19.

<sup>74</sup> A. M. Bateman, *ibid.*, 1932, **27**, 52; R. Juza and W. Biltz, *Z. anorg. Chem.*, 1930, **190**, 161.

<sup>75</sup> H. Reinhold and K. Schmitt, *Z. physikal. Chem.*, 1939, *B*, **44**, 75.

excess of iron.<sup>66</sup>  $\overline{\text{FeSb}}$  exists at the ordinary temperature only over the range  $\text{Fe}_{1.15}\text{Sb}$  to  $\text{Fe}_{1.25}\text{Sb}$ ,<sup>67</sup> and the maximum melting point corresponds roughly to  $\text{Fe}_{1.25}\text{Sb}$ .<sup>68</sup> The ideal formula, however, is established by the NiAs lattice type.  $\overline{\text{Sb}}$ <sup>69</sup> and  $\overline{\text{NiBi}}$ <sup>70</sup> similarly have an existence range with excess of metal. Stoichiometric  $\text{FeSi}_2$  is non-existent; <sup>71</sup> the phase of maximum melting point has a 20% deficiency of cations, due possibly to substitutional solid solution.<sup>72, 2</sup>

(IV) *Oxides*.—Non-stoichiometric phases have been reported in the systems listed below but, as will be evident, finality has not been reached in a number of instances. Possible reasons for over-estimating ranges of existence have already been indicated.

*Titanium*.<sup>73</sup>  $\alpha$ -Phase,  $\overline{\text{TiO}_2}$ ,  $\text{TiO}_{2.00}$ — $\text{TiO}_{1.90}$ ;  $\beta$ -phase, lower symmetry than rutile,  $\text{TiO}_{1.80}$ — $\text{TiO}_{1.70}$ ;  $\gamma$ -phase,  $\overline{\text{Ti}_2\text{O}_3}$ , corundum type,  $\text{TiO}_{1.56}$ — $\text{TiO}_{1.46}$ ;  $\delta$ -phase,  $\overline{\text{TiO}}$ , NaCl type,  $\text{TiO}_{1.35}$ — $\text{TiO}_{0.6}$ ; in addition, the metal takes up about 42 atoms % of oxygen in interstitial solid solution.<sup>74</sup> The  $\overline{\text{TiO}}$  phase is of interest as showing how, in a structure with a very high degree of Schottky lattice disorder, stoichiometric variation arises from the unbalance of anion and cation holes.

Composition.	$\text{TiO}_{1.33}$	$\text{TiO}_{1.12}$	$\text{TiO}_{1.00}$	$\text{TiO}_{0.69}$
Lattice sites occupied : Ti, % .....	74	81	85	96
O, % .....	98	91	85	66

*Zirconium*. Oxides have not yet been investigated, but the metal takes up oxygen interstitially to at least  $\text{ZrO}_{0.4}$ .<sup>75</sup>

*Vanadium*.  $\overline{\text{V}_2\text{O}_3}$ , corundum type, extends from  $\text{VO}_{1.35}$  to  $\text{VO}_{1.5}$  approx.;  $\overline{\text{VO}}$ , NaCl type, from  $\text{VO}_{1.3}$  to  $\text{VO}_{0.9}$ , though the range of existence is much narrower at low temperatures.<sup>76</sup>

*Niobium*. Oxides have very limited ranges of composition (cf. the less ready variability of valency of Nb as compared with V).  $\text{Nb}_2\text{O}_5$  has a probable range  $\text{NbO}_{2.5}$ — $\text{NbO}_{2.4}$ ;  $\text{NbO}_2$ ,  $\text{NbO}$  (cf.  $\overline{\text{TiO}}$  and  $\overline{\text{VO}}$ ) no detectable ranges, though the  $\text{NbO}$  structure is of a unique defect lattice type.<sup>77</sup>

Results for *chromium* oxides are conflicting, and need revision. A. Cameron, E. H. Harbard, and A. King<sup>78</sup> found bivariant equilibria in the

<sup>66</sup> L. H. Bauer and H. Bermann, *Amer. Min.*, 1927, **12**, 39; M. J. Buerger, *ibid.*, 1934, **19**, 37.

<sup>67</sup> Ref. (9).

<sup>68</sup> R. Vogel and W. Dannöhl, *Arch. Eisenhüttenw.*, 1934, **8**, 39.

<sup>69</sup> E. S. Makarov, *Ann. Sect. d'Anal. Phys.-Chim.*, 1943, **16**, No. 1; *A.*, 1943, **I**, 15.

<sup>70</sup> G. Hägg and G. Funke, *Z. physikal. Chem.*, 1929, **B**, **6**, 272.

<sup>71</sup> G. Phragmen, *J. Iron Steel Inst.*, 1926, **114**, 397; M. Bamberger, O. Einerl, and J. Nussbaum, *Stahl u. Eisen*, 1925, **45**, 141.

<sup>72</sup> J. L. Haughton and M. L. Becker, *J. Iron Steel Inst.*, 1930, **121**, 315.

<sup>73</sup> P. Ehrlich, *Z. Elektrochem.*, 1939, **45**, 362.

<sup>74</sup> *Idem*, *Z. anorg. Chem.*, 1941, **247**, 53.

<sup>75</sup> J. H. de Boer and J. Fast, *Rec. Trav. chim.*, 1940, **59**, 161.

<sup>76</sup> W. Klemm and L. Grimm, *Z. anorg. Chem.*, 1942, **250**, 42.

<sup>77</sup> G. Brauer, *ibid.*, 1941, **243**, 1.

ranges  $\text{CrO}_{1.7}$ — $\text{CrO}_{1.9}$ ,  $\text{CrO}_{2.2}$ — $\text{CrO}_{2.6}$ , covering the range of complex oxides reported by earlier workers.<sup>79</sup> A. Michel and J. Bénard,<sup>80</sup> however, do not find these phases, but report that  $\overline{\text{Cr}_2\text{O}_3}$  has an upper limit of composition about  $\text{CrO}_{1.56}$ .

Lower oxides of *molybdenum* have long been a matter of disagreement. According to G. Hägg and A. Magnéli,<sup>81</sup> the system is similar to that of the tungsten oxides, with  $\beta$ - and  $\beta'$ -phases, roughly  $\text{MoO}_{2.92}$ — $\text{MoO}_{2.85}$ ;  $\gamma$ -phase  $\text{MoO}_{2.85}$ — $\text{MoO}_{2.72}$ ;  $\delta$ -phase  $\text{MoO}_2$ .

*Tungsten.* O. Glemser and H. Sauer<sup>82</sup> find:  $\alpha$ -phase  $\text{WO}_3$ — $\text{WO}_{2.95}$ ;  $\beta$ -phase  $\text{WO}_{2.92}$ — $\text{WO}_{2.88}$ ;  $\gamma$ -phase  $\text{WO}_{2.75}$ — $\text{WO}_{2.65}$  ( $\overline{\text{W}_4\text{O}_{11}}$ , with  $X$ -ray diagram identical with the  $\text{W}_4\text{O}_{11}$  of F. Ebert and H. Flasch);<sup>83</sup>  $\delta$ -phase  $\text{WO}_{2.05}$ — $\text{WO}_{2.00}$ . Hägg and Magnéli substantially confirm these results.<sup>81</sup> Closely related are the interesting tungsten bronzes,  $\text{Na}_x\text{WO}_3$ , etc., of which the stoicheiometric compounds ( $x = 1$ ) apparently do not exist; the cubic sodium bronzes ( $x = 0.95$ — $0.30$ ) are of defective perovskite type, giving place (for  $x = 0.3$ — $0.2$ ) to structures of lower symmetry.<sup>84</sup> The tungsten blues, and the hydrogen-containing compounds studied by Ebert and Flasch<sup>83</sup> and by O. Glemser and H. Sauer<sup>82, 85</sup> appear similar in constitution.

*Uranium.* It seems clearly established that at elevated temperatures stoicheiometric  $\text{UO}_3$  is unstable, and the  $\overline{\text{U}_3\text{O}_8}$  phase is of widely variable composition.<sup>86</sup>

*Manganese.* Numerous oxides intermediate between  $\text{Mn}_2\text{O}_3$  and  $\text{MnO}_2$  have been reported, but their individuality is questionable: if they are not mixtures, a non-stoicheiometric phase seems likely. A. Simon and F. Feher<sup>87</sup> inferred the existence of such a phase from tensimetric studies, but other workers<sup>88</sup> appear agreed that pyrolusite has, at the most, only a small composition range, although it usually contains less oxygen than corresponds to  $\text{MnO}_{2.00}$ . However, at least three modifications of  $\text{MnO}_2$  appear to exist.<sup>89, 90</sup> Wet methods of preparation [*e.g.*, oxidation of  $\text{Mn}(\text{OH})_2$  or  $\text{MnO}\cdot\text{OH}$ ] can produce hydrous oxides of variable composition (but few structural defects) through double substitution of  $\text{Mn}^{3+}$  for  $\text{Mn}^{4+}$  and  $\text{OH}^-$

<sup>78</sup> J., 1939, 55; S. S. Bhatnagar, A. Cameron, E. H. Harbard, P. D. Kapur, A. King, and B. Prakash, *J.*, 1939, 1433.

<sup>79</sup> Cf. A. Simon and T. Schmidt, *Z. anorg. Chem.*, 1926, **153**, 191.

<sup>80</sup> *Bull. Soc. chim.*, 1943, **10**, 315.

<sup>81</sup> *Arkiv Kemi Min. Geol.*, 1944, **19**, A, No. 2; *A.*, 1946, **I**, 144.

<sup>82</sup> *Z. anorg. Chem.*, 1943, **252**, 144.

<sup>83</sup> *Ibid.*, 1934, **217**, 95; 1935, **226**, 65.

<sup>84</sup> G. Hägg, *Nature*, 1935, **135**, 874; *Z. physikal. Chem.*, 1935, **B**, **29**, 192.

<sup>85</sup> *Z. anorg. Chem.*, 1943, **252**, 160.

<sup>86</sup> W. Biltz and H. Müller, *ibid.*, 1927, **163**, 257.

<sup>87</sup> *Z. Elektrochem.*, 1932, **38**, 137.

<sup>88</sup> M. Le Blanc and G. Wehner, *Z. physikal. Chem.*, 1934, **A**, **168**, 59; C. Drucker and R. Hüttner, *ibid.*, 1928, **131**, 237; P. Dubois, *Ann. Chim.*, 1936, **5**, 411.

<sup>89</sup> P. Dubois, *loc. cit.*, ref. (88); O. Glemser, *Ber.*, 1939, **72**, 1879.

<sup>90</sup> W. F. Cole, A. D. Wadsley, and A. Walkley, private communication.

for  $O^{2-}$ .<sup>90, 91</sup> It is reported that one modification of  $Mn_2O_3$  takes up excess oxygen to  $MnO_{1.58}$  at least.<sup>92</sup>

**Iron.** Two ranges of non-stoichiometric oxides are of importance in metallurgy. (I) R. Schenck and T. Dingmann<sup>93</sup> first reported that the  $FeO$  (wüstite) phase, stable only above  $580^\circ$ , invariably contains a stoichiometric excess of oxygen which represents an excess of vacant cation sites.<sup>8</sup> At  $1400^\circ$  the range of composition extends from  $FeO_{1.055}$  to  $FeO_{1.19}$ .<sup>94</sup> Although his interpretation cannot be accepted, the work of J. Bénard<sup>95</sup> has shown that in the oxidation of iron at high temperatures the primary product is the  $FeO$  phase, with a continuous composition gradient from the iron-rich limit to the oxygen-rich limit. A means is thereby provided for a continuous diffusion of iron through the oxide film to the  $FeO-O_2$  interface, as envisaged by K. Fischbeck.<sup>96</sup> (II) G. Hägg<sup>97</sup> showed that  $Fe_3O_4$  and the  $\gamma$ - $Fe_2O_3$  defective spinel structure represented the limits of one phase of variable composition. At high temperatures, where  $\gamma$ - $Fe_2O_3$  is unstable, the phase relations are still uncertain. R. C. Sosman and J. C. Hostetter<sup>98</sup> concluded that  $\alpha$ - $Fe_2O_3$  and  $Fe_3O_4$  had extended ranges of existence towards lower and higher oxygen contents respectively, and J. C. White<sup>99</sup> appears to confirm this substantially. Later work by Sosman *et al.*<sup>100</sup> indicated that neither  $Fe_2O_3$  nor  $Fe_3O_4$  was appreciably variable in composition.

**Cobalt.** M. Le Blanc and E. Möbius<sup>101</sup> and M. Watanabe<sup>102</sup> report that  $CoO$  and  $Co_3O_4$  can each take up a substantial stoichiometric excess of oxygen.

**Nickel.** Black  $NiO$  was found by M. Le Blanc and H. Sachse<sup>103</sup> to contain an excess of oxygen, although a homogeneous phase. According to W. Klemm and E. Hass, stoichiometric  $NiO$  is metastable, breaking up into  $NiO_{1.005} + Ni$ .<sup>104</sup>

**Copper.**  $CuO$  is apparently stoichiometric, but the much studied semi-conducting properties of  $Cu_2O$  depend on a small excess of oxygen, which is present in true equilibrium with the gaseous phase at high temperatures, as envisaged by theory. Measurements by C. Wagner and H. Hammen<sup>105</sup> give, in equilibrium with 0.7 mm. of  $O_2$  at  $1000^\circ$ ,  $Cu_2O_{1.00052}$ ; with 33 mm. of  $O_2$ ,  $Cu_2O_{1.00114}$ .

<sup>91</sup> W. Feitknecht and W. Marti, *Helv. Chim. Acta*, 1945, **28**, 129, 149.

<sup>92</sup> (a) M. Blumenthal, *Bull. Soc. chim.*, 1933, **53**, 1418; (b) C. B. Holtermann, *Ann. Chim.*, 1940, **14**, 121.

<sup>93</sup> *Z. anorg. Chem.*, 1927, **166**, 113.

<sup>94</sup> L. S. Darken and R. W. Gurry, *J. Amer. Chem. Soc.*, 1945, **67**, 1398.

<sup>95</sup> *Ann. Chim.*, 1939, **12**, 5; *Compt. rend.*, 1943, **217**, 77.

<sup>96</sup> *Z. Metallk.*, 1932, **24**, 313; *Metallwirts.*, 1935, **14**, 733.

<sup>97</sup> *Z. physikal. Chem.*, 1935, B, **29**, 95.

<sup>98</sup> *J. Amer. Chem. Soc.*, 1916, **38**, 807.

<sup>99</sup> Iron and Steel Inst., Carnegie Schol. Mem., 1938, **27**, 1.

<sup>100</sup> *Amer. J. Sci.*, 1935, **30**, 239.

<sup>101</sup> *Z. physikal. Chem.*, 1929, A, **142**, 151.

<sup>102</sup> *Sci. Rep. Tôhoku*, 1934, **23**, 89; A., 1934, 599.

<sup>103</sup> *Z. Elektrochem.*, 1926, **32**, 58, 204.

<sup>104</sup> *Z. anorg. Chem.*, 1934, **219**, 82.

<sup>105</sup> *Z. physikal. Chem.*, 1938, B, **40**, 197.

*Zinc.* Although the composition of ZnO is not measurably variable, its semiconducting properties show that the colour change on heating is associated with a loss of oxygen and the presence of a minute excess of zinc.<sup>106</sup> The red ZnO obtained by A. Kutzelnigg<sup>107</sup> has been shown by A. Greenstone and W. Ehret<sup>108</sup> to contain up to 0.02% excess of zinc, but may well be thermodynamically highly unstable. *Cadmium* oxide probably has at least a similar range of composition,<sup>109</sup> and the reversible colour changes of other oxides (In<sub>2</sub>O<sub>3</sub>, CeO<sub>2</sub>) can probably be interpreted similarly. Finality as to the oxides of *lead* has certainly not yet been reached. Pb<sub>3</sub>O<sub>4</sub> seems to be a closely stoichiometric compound, but M. Le Blanc and E. Eberius<sup>110</sup> concluded that PbO, PbO<sub>2</sub>, and another intermediate oxide were all non-stoichiometric. It now appears<sup>111</sup> that PbO<sub>2</sub> has only a narrow range, perhaps from PbO<sub>1.95</sub> to PbO<sub>2.00</sub>; it is probably not obtainable without constitutional water and the limited stoichiometric variability could arise from replacement of 2O<sup>2-</sup> by 2OH<sup>-</sup>, Pb<sup>4+</sup> by Pb<sup>2+</sup>, in the ideal structure (cf. MnO<sub>2</sub>). By degradation of PbO<sub>2</sub>, or by reaction of PbO with oxygen, two definitely non-stoichiometric intermediate oxides may be formed, but there is no agreement as to their nature. Byström's  $\alpha$ -PbO<sub>x</sub> with the range PbO<sub>1.5</sub>—PbO<sub>1.67</sub> may correspond with the non-stoichiometric<sup>112</sup> or stoichiometric<sup>113</sup> Pb<sub>5</sub>O<sub>8</sub> or Pb<sub>7</sub>O<sub>11</sub><sup>92b</sup> phases of other workers (although there is no agreement as to the symmetry of this phase). Byström's  $\beta$ -PbO<sub>x</sub> (PbO<sub>1.47</sub>—PbO<sub>1.51</sub>) probably corresponds to G. L. Clark and R. Rowan's PbO<sub>x</sub>. However, the discrepancies between different workers are not to be reconciled.

*Antimony.* The oxides provide an instructive instance of false conclusions drawn from tensimetric measurements, indicative of a phase of continuous composition between Sb<sub>2</sub>O<sub>3</sub> and Sb<sub>2</sub>O<sub>5</sub><sup>114</sup> and apparently confirmed by X-ray measurements.<sup>115</sup> Later work<sup>116</sup> has put a completely different interpretation on the facts, and provides no evidence of stoichiometrically variable antimony oxides.

(V) *Halides.*—The electrical conductivity of cuprous iodide is strongly dependent on the pressure of iodine in equilibrium with the solid compound. This, as first shown by K. Bädcker,<sup>117</sup> takes up a stoichiometric excess of

<sup>106</sup> Ref. (38). <sup>107</sup> *Z. anorg. Chem.*, 1932, **208**, 23; 1934, **221**, 116.

<sup>108</sup> *J. Amer. Chem. Soc.*, 1943, **65**, 872.

<sup>109</sup> R. Faivre, *Ann. Chim.*, 1944, **19**, 58; H. H. v. Baumbach and C. Wagner, *Z. physikal. Chem.*, 1933, **B**, **22**, 199.

<sup>110</sup> *Ibid.*, 1932, **A**, **160**, 69.

<sup>111</sup> A. Byström, *Arkiv Kemi Min. Geol.*, 1945, **20**, **A**, No. 11.

<sup>112</sup> G. L. Clark and R. Rowan, *J. Amer. Chem. Soc.*, 1941, **63**, 1305.

<sup>113</sup> F. Fischer and H. Ploetze, *Z. anorg. Chem.*, 1912, **75**, 1.

<sup>114</sup> A. Simon and E. Thaler, *ibid.*, 1927, **162**, 253.

<sup>115</sup> U. Dehlinger, *Z. physikal. Chem.*, 1929, **B**, **6**, 127; U. Dehlinger and R. Glocker, *Z. anorg. Chem.*, 1927, **165**, 41.

<sup>116</sup> K. Dählström and A. Westgren, *ibid.*, 1937, **235**, 153; K. Dählström, *ibid.*, 1938, **239**, 57.

<sup>117</sup> *Ann. Physik*, 1907, **22**, 749; 1909, **29**, 566; *Physikal. Z.*, 1908, **9**, 431; 1912, **13**, 1080; K. Nagel and C. Wagner, *Z. physikal. Chem.*, 1933, **B**, **25**, 71.

iodine, up to the composition  $\text{CuI}_{1.0045}$ . The  $\overline{\text{CuI}}$  system is one of the few for which ( $p, T, X$ ) equilibrium data can be correlated properly with measurements of semiconducting properties.<sup>118</sup>

(VI) *Ternary Compounds*.—Distinction between non-stoichiometric compounds and mixed-crystal phases is here more arbitrary, since in addition to subtractive and interstitial types of solid solution, there is the possibility of "anomalous" solid solutions—also involving the creation of vacant lattice sites or interstitial atoms—of the kind exemplified by the  $\gamma\text{-Al}_2\text{O}_3\text{-MgAl}_2\text{O}_4$  phase,<sup>28</sup> and the defective fluorite-type solid solutions studied by Zintl *et al.*<sup>119</sup> However, certain classes of ternary compound have been described which are inherently non-stoichiometric, *e.g.*, the tungsten bronzes already mentioned.<sup>84</sup> Sillén and his co-workers have recently described a number of double oxides and oxy-halides of bismuth with bivalent metals in which, by variation in the  $\text{M}^{3+}:\text{M}^{2+}$  cation ratio, either (i) the cation lattice remains complete, but a variable proportion of oxygen sites is vacant, or (ii) the anion lattice is perfect, but the number of cations in the structure is variable. Rational formulæ cannot always be assigned to "idealised" compounds. Of type (i) are the double oxides  $\text{M}^{\text{II}}_{2-x}\text{Bi}_{2-2x}\text{O}_{3-x}$ —*e.g.*,  $\text{Pb}_{1.2}\text{Bi}_{0.8}\text{O}_{2.40}$  to  $\text{Pb}_{0.64}\text{Bi}_{1.36}\text{O}_{2.68}$ ,<sup>120</sup> and analogous compounds of cadmium<sup>121</sup> and the alkaline earths.<sup>122</sup> The oxyhalides of bismuth with calcium,<sup>123</sup> cadmium,<sup>124</sup> and other bivalent metals are of type (ii), and exemplify some very interesting structural principles; the inherently non-stoichiometric phases  $\text{M}^{\text{II}}_{2-3x}\text{Bi}_{1+2x}\text{O}_2\text{X}_3$ ,  $\text{M}^{\text{II}}_{2-3x}\text{Bi}_{3+2x}\text{O}_4\text{X}_5$ ,  $\text{M}^{\text{II}}_{2-3x}\text{Bi}_{5+2x}\text{O}_6\text{X}_7$  ( $\text{X} = \text{Cl}, \text{Br}$ ) have been described. According to C. Brosset,<sup>125</sup> potassium cryolite, ideally  $\text{K}_3\text{AlF}_6$ , may vary in composition through replacement of  $\text{AlF}_6^{3-}$  groups by  $[\text{AlF}_5(\text{H}_2\text{O})]^{2-}$  groups, with corresponding omission of  $\text{K}^+$  cations (up to 3%) from the structure. A range of homogeneity has also been assigned to the alkali tantalates and niobates.<sup>126</sup> It is likely *a priori* that the ternary sulphides, etc., will be variable in composition, but few systems have been closely studied. Chalcopyrite appears definitely non-stoichiometric, with the limiting composition  $\text{CuFeS}_{1.94}$ .<sup>127</sup>

*Rôle of Non-stoichiometric Phases in the Reactions of Solids*.—Reactions between solids, or between solid and fluid substances, take place at the interface between the reactants. Transport of reactant to this interface must take place, in general, by diffusion through the solid product of reaction, and this may be the rate-determining process in the reaction. The

<sup>118</sup> R. J. Maurer, *J. Chem. Physics*, 1945, **13**, 321.

<sup>119</sup> *Z. anorg. Chem.*, 1939, **240**, 145, 150; 1939, **242**, 79.

<sup>120</sup> L. G. Sillén and B. Aurivillois, *Naturwiss.*, 1939, **27**, 388; *Z. Krist.*, 1939, **101**, 483.

<sup>121</sup> L. G. Sillén and B. Sillén, *Z. physikal. Chem.*, 1941, **B**, **49**, 27.

<sup>122</sup> B. Aurivillois, *Arkiv Kemi Min. Geol.*, 1943, **16**, A, No. 17.

<sup>123</sup> L. G. Sillén and A. S. Gjöring-Husberg, *Z. anorg. Chem.*, 1941, **248**, 121, 135.

<sup>124</sup> L. G. Sillén, *ibid.*, 1941, **246**, 331.

<sup>125</sup> *Arkiv Kemi Min. Geol.*, 1946, **21**, A, No. 9.

<sup>126</sup> F. Halla, A. Neth, and F. Windmaisser, *Z. Krist.*, 1942, **104**, 161.

<sup>127</sup> H. E. Merwin and R. H. Lombard, *Econ. Geol.*, 1937, **32**, 203.

mechanism of diffusion, and of ionic conduction, in polar solids can be interpreted in terms of the presence and migration of lattice defects,<sup>33</sup> and departure from stoichiometry, by controlling the concentration of interstitial ions or vacant sites, affects the diffusion coefficient  $D$ . This applies both to ionic conductivity or diffusion along a concentration gradient and to self-diffusion,<sup>128</sup> as has recently been shown experimentally.<sup>129</sup> Where only one ion (most frequently the cation) is mobile,  $D$  is a minimum for the stoichiometric crystal, in the case of a crystal with Frenkel defects, or increases monotonically with stoichiometric excess of non-metal for the case of Schottky defects. In reactions, stoichiometric variations enable a composition gradient to be set up through the layer of reaction product. C. Wagner<sup>130</sup> has derived a quantitative theory for "tarnish" reactions, which proceed by continuous migration of cations to the solid-gas interface [cf. ref. (96)], and has extended it to include reactions between solids—*e.g.*, double salt, spinel, and silicate formation.<sup>131</sup> The acceleration of such processes by conditions producing small deviations from stoichiometry has been demonstrated for the formation of  $\text{MgAl}_2\text{O}_4$ .<sup>132</sup> Both  $\text{Al}_2\text{O}_3$  and  $\text{MgO}$  are metal-excess conductors at high temperatures, and their union proceeds markedly faster in vacuum or in hydrogen than in air. Such factors may have considerable significance in ceramic processes.<sup>133</sup>

There is some evidence that non-stoichiometric phases can be formed under non-equilibrium conditions, as intermediate stages in the formation or dissociation of solid compounds. For instance, brucite formed by slow oxidation of magnesium in moist oxygen,<sup>134</sup> or partially dissociated silver oxide<sup>135</sup> is stated to contain an excess of metal. Analogous cases are on record.<sup>107, 136</sup>

In a broad sense, the non-stoichiometric character of a solid may be associated with the mechanism of catalysis in heterogeneous reactions. Thus, C. Wagner and K. Hauffe<sup>137</sup> have deduced the rate-determining step in the nickel-oxide-catalysed reactions  $2\text{CO} + \text{O}_2 = 2\text{CO}_2$ ,  $2\text{N}_2\text{O} = 2\text{N}_2 + \text{O}_2$ , from the composition of the oxide catalyst (as shown by its electronic conductivity) in the stationary state. Similar observations have been made for the  $\text{H}_2 + \text{S} \longrightarrow \text{H}_2\text{S}$  reaction catalysed by silver sulphide,<sup>138</sup> and the mechanism of the catalysed water-gas reaction<sup>139</sup> and the ammonia

<sup>128</sup> C. Wagner, *Z. physikal. Chem.*, 1931, **Bodenstein Festb.**, 177.

<sup>129</sup> J. S. Anderson and J. R. Richards, *J.*, 1946, 537.

<sup>130</sup> *Z. physikal. Chem.*, 1933, **B**, **21**, 25; 1936, **B**, **32**, 447; *Angew. Chem.*, 1936, **49**, 735.

<sup>131</sup> C. Wagner, *Z. physikal. Chem.*, 1936, **B**, **34**, 309, 317.

<sup>132</sup> H. C. Castel, S. Dilnot and M. Warrington, *Nature*, 1944, **153**, 653.

<sup>133</sup> Cf. J. A. Hedvall, *Die Chemie*, 1942, **55**, 334; *Trans. Chalmers Univ. Technology, Göteborg*, 1942, No. 15.

<sup>134</sup> R. Faivre and A. Michel, *Compt. rend.*, 1939, **208**, 1008.

<sup>135</sup> R. Faivre, *ibid.*, 1940, **210**, 398.

<sup>136</sup> E. I. Mokeeva and N. I. Mokeeva, *J. Physical Chem. Russ.*, 1941, **15**, 686.

<sup>137</sup> *Z. Electrochem.*, 1938, **44**, 172.

<sup>138</sup> H. Reinhold, W. Appel, and P. Frisch, *Z. physikal. Chem.*, 1939, **A**, **184**, 273.

<sup>139</sup> E. Doehlmann, *Z. Elektrochem.*, 1938, **44**, 178.



synthesis<sup>5</sup> have been discussed from a similar standpoint. A further influence upon the surface properties of solids is shown by the dependence of adsorptive properties of metallic sulphides upon small variations of stoichiometric composition.<sup>140</sup>

J. S. A.

## 2. COMPLEX COMPOUNDS OF THE PLATINUM METALS.

In 1944<sup>1</sup> it was observed that platinum metal complexes have recently received special attention particularly by Russian workers, but it was found necessary to defer discussion of their work to a later report, and it is this work which forms the bulk of the present review.

### *Platinum.*

*Olefin Complexes.*—The complexes formed by metallic salts and olefins were discovered before 1830<sup>2</sup> but no satisfactory structure has yet been assigned to them. Since the last review of this subject in 1936 a comprehensive account of these compounds by R. N. Keller<sup>3</sup> and a considerable volume of work by Hel'man and his co-workers have been published. Platinum salts form the best-known and most stable complexes, so work has been limited almost entirely to the platinum series. Palladium complexes are less stable and recent attempts to obtain cobalt and nickel complexes were unsuccessful.<sup>4</sup> Typical members of the series are  $K[Pt C_2H_4 Cl_3]$ ,  $[(Pt C_2H_4 Cl_2)_2]$ ,  $[Pt C_2H_4 py Cl_2]$  and the most recently added member  $[Pt C_2H_4 NH_3 py Cl]NO_3$ .<sup>5</sup> The anionic complex is very much more stable than the cationic complex, and attempts to obtain two mono-olefins attached to one platinum atom have so far failed.<sup>6, 16</sup>

Unsaturated molecules behave similarly to ammonia and occupy only one co-ordination place round the platinum atom, but they differ from ammonia and pyridine in their directing influence on substituents entering a complex which already contains an olefin. They labilise the group in the *trans*-position so that, in preference to the *cis*-groups, it is replaced by the entering substituent. This difference is illustrated by comparing the reactions (A) and (B) with the analogous reactions (C) and (D).<sup>7, 8</sup>

Whilst the products (I) and (II) are identical, but different from Jorgensen's<sup>9</sup>  $[Pt(NH_3) py Cl_2]$ , products (III) and (IV) are isomeric. (I), (II), and (III) are claimed to be *cis*-isomers, but (IV) is claimed to be the *trans*-

<sup>140</sup> J. A. Hedvall and S. Nord, *Z. Elektrochem.*, 1943, **49**, 467.

<sup>1</sup> *Ann. Reports*, 1944, **41**, 98.

<sup>2</sup> W. C. Zeise, *Mag. Pharm.*, 1830, **35**, 105.

<sup>3</sup> *Chem. Reviews*, 1941, **28**, 229.

<sup>4</sup> A. D. Hel'man and I. B. Litvak, *Ann. Secteur platine, Inst. chim. gén. (U.S.S.R.)*, 1939, **16**, 29.

<sup>5</sup> A. D. Hel'man and E. A. Meilakh, *Compt. rend. Acad. Sci. U.R.S.S.*, 1946, **51**, 207.

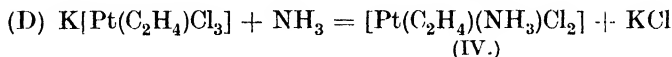
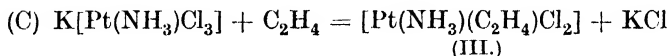
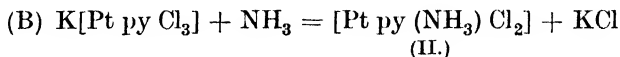
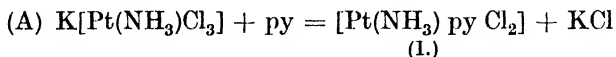
<sup>6</sup> A. D. Hel'man, *ibid.*, 1939, **23**, 532.

<sup>7</sup> I. I. Chernyaev and A. D. Hel'man, *Ann. Secteur platine, Inst. chim. gén. (U.S.S.R.)*, 1938, **15**, 5.

<sup>8</sup> A. D. Hel'man, *Compt. rend. Acad. Sci. U.R.S.S.*, 1939, **22**, 107.

<sup>9</sup> S. M. Jørgensen, *J. pr. Chem.*, 1886, **33**, 489.

isomer, and the analogously prepared pyridine complex  $[\text{Pt}(\text{C}_2\text{H}_4)\text{py Cl}_2]$  reacts with pyridine to give *trans*- $[\text{Pt py}_2 \text{Cl}_2]$ .<sup>10</sup>



Reactions (C) and (D) have been shown to be general and have been applied to obtain similar isomers containing other olefins and carbon monoxide instead of ethylene as well as bromine instead of chlorine.<sup>11, 12</sup> By further adaption isomers containing four different groups attached to the platinum atom have been obtained, *e.g.*,  $[\text{Pt}(\text{C}_2\text{H}_4)(\text{NH}_3)\text{ClBr}]$ .<sup>13</sup>

It was shown by J. S. Anderson<sup>14, 15</sup> that the stability of the ethylene complexes of  $\text{PtCl}_2$  was altered markedly by substitution of other univalent radicals in place of chlorine and also by substitution in the ethylene molecule itself. This work has been repeated and extended by Russian workers, who have found that the amine substituent in  $[\text{Pt}(\text{C}_2\text{H}_4)\text{am Cl}_2]$  causes a decrease of stability in the order: <sup>10</sup> am = quinoline > pyridine > ammonia > thiourea. They confirm that the stability decreases as chlorine is replaced in the order:  $\text{Cl} > \text{Br} > \text{I} > \text{NO}_2 > \text{CNS} > \text{CN}$ , but differ from Anderson in placing styrene higher than ethylene in the stability of its complexes. They find the following order of stabilities: <sup>16, 17</sup>  $\text{NO} > \text{CO} > \text{styrene} > \text{butadiene} \sim \text{C}_2\text{H}_4 > \text{C}_3\text{H}_6 \sim \text{C}_4\text{H}_8$ .

This work is somewhat qualitative as no allowance is made for the relative volatilities and solubilities of the various olefins. The replacements of unsaturated molecules were effected by reaction of the appropriate unsaturated substance Un with a dilute acid solution of the salt  $\text{K}[\text{PtUnCl}_3]$ , then trying the reverse replacement Un' into  $\text{K}[\text{PtUnCl}_3]$ . Sometimes it was found that both replacements occurred, *e.g.*, with propylene and butylene.

Besides the above series of stabilities two interesting facts emerged; when attempts were made to replace CO by NO in  $\text{py H}[\text{Pt}(\text{CO})\text{Cl}_3]$  in very acid solution by passing NO through it for two months, the platinum was oxidised and  $(\text{py H})_2[\text{PtCl}_6]$  was isolated; <sup>16</sup> and also the reaction of ethylene with the  $[\text{PtCl}_4]^-$  ion in dilute acid solution is catalysed by propylene.<sup>17</sup>

<sup>10</sup> I. I. Chernyaev and A. D. Hel'man, *Ann. Secteur platine, Inst. chim. gén. (U.S.S.R.)*, 1937, **14**, 77.

<sup>11</sup> A. D. Hel'man, *Compt. rend. Acad. Sci. U.R.S.S.*, 1937, **16**, 351.

<sup>12</sup> A. D. Hel'man and M. Bauman, *ibid.*, 1938, **18**, 645.

<sup>13</sup> A. D. Hel'man, *ibid.*, 1943, **38**, 310. <sup>14</sup> J., 1934, 971. <sup>15</sup> J., 1936, 1042.

<sup>16</sup> A. D. Hel'man, *Compt. rend. Acad. Sci. U.R.S.S.*, 1941, **32**, 347.

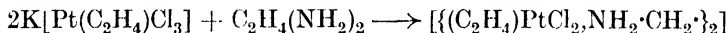
<sup>17</sup> A. D. Hel'man, *ibid.*, 1938, **20**, 307.

By passage of a mixture of propylene and ethylene into an acid solution of potassium chloroplatinite for four days, a 52% yield of Zeise's salt free from the propylene salt was obtained, whereas pure ethylene in the same time would have given only a 15% yield. It is suggested that the greater solubility of propylene leads to a more rapid reaction with the  $[\text{PtCl}_4]^-$  ion, yielding  $[\text{Pt}(\text{C}_3\text{H}_6)\text{Cl}_3]^-$  as intermediate from which the propylene is rapidly evicted by the ethylene.

It is interesting that, although CO readily replaces all the olefins from ions of the type  $[\text{Pt Un Cl}_3]^-$ , the  $[\text{Pt}(\text{CO})\text{Cl}_3]^-$  ion produced is comparatively unstable, being decomposed by water except in strongly acid solution. On the other hand, the NO complexes are exceptionally stable, yet NO replaces the olefins only very slowly.

It appears that the diolefins, butadiene and diallyl, do not form chelate complexes but each double bond reacts with a different platinum atom.<sup>6, 16</sup>

Attempts to obtain chelate compounds by using ethylenediamine led to no greater success :<sup>18</sup>



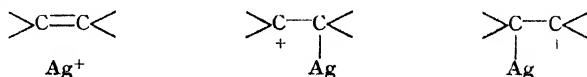
The structure of these complex compounds remains unsolved.\* Hel'man<sup>19</sup> has suggested that the ethylene molecule undergoes an electromeric change in the presence of the platinum-containing ion, and the carbon atom with a deficiency of electrons accepts two electrons from the platinum atom, presumably from a  $5d$  orbital, thus raising the platinum to the platinic state. The other carbon atom now donates its electrons to the platinum atom, forming a four-electron bond between the ethylene molecule and the platinum atom. In support of this the electrometric titrations of  $\text{K}[\text{Pt}(\text{NH}_3)\text{Cl}_3]$ ,  $\text{NH}_4[\text{Pt}(\text{C}_2\text{H}_4)\text{Cl}_3]$ ,  $\text{K}_2[\text{PtCl}_3(\text{C}_4\text{H}_6)\text{PtCl}_3]$ , and  $\text{NH}_4[\text{Pt}(\text{NH}_3)\text{Cl}_5]$  were compared in acid solution using 0.1N-permanganate to effect the oxidation. Oxidation occurred only when  $\text{K}[\text{Pt}(\text{NH}_3)\text{Cl}_3]$  was titrated;<sup>20</sup> also the initial potential of the solution of  $\text{K}[\text{Pt}(\text{C}_2\text{H}_4)\text{Cl}_3]$  was 650 mv., and of the above butadiene analogue 700 mv. as compared with 660 mv. for  $\text{NH}_4[\text{Pt}(\text{NH}_3)\text{Cl}_5]$  and 520 mv. for  $\text{NH}_4[\text{Pt}(\text{NH}_3)\text{Cl}_3]$ . These results support the suggestion that platinum in the olefin complexes is in the platinic state, but this point deserves further investigation for it would appear that we

<sup>18</sup> *Doklady Akad. Nauk. S.S.S.R.*, 1943, **38**, 272.

<sup>19</sup> A. D. Hel'man, *Compt. rend. Acad. Sci. U.R.S.S.*, 1939, **24**, 549.

<sup>20</sup> A. D. Hel'man and D. I. Ryabchikov, *ibid.*, 1941, **33**, 462.

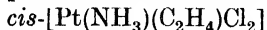
\* Since this report was completed, A. D. Walsh (*Nature*, 1947, **159**, 165; *J.*, 1947, 89) has pointed out that the ionisation potential of the  $\pi$  electrons of ethylene is 10.45 v. as against 10.8 v. for the lone pair electrons of ammonia and suggested that the  $\pi$  electrons should be capable of donation to suitable atoms or groups thus binding together three nuclei. This view is closely allied to that of Winstein and Lucas (*J. Amer. Chem. Soc.*, 1938, **60**, 836) who proposed a resonance of the three structures:



after their study of the silver ion complexes.

have the unusual and somewhat loose combination of two reducing substances to yield a product resistant to oxidation by permanganate.

Bokiï<sup>21, 22, 23</sup> has attempted to obtain the structure of



by X-ray methods and claims that the substance is dimeric, with a Pt-Pt bond of length 1.4 Å. Each platinum atom is surrounded in a distorted octahedron by the other platinum atom, two carbon atoms, two chlorine atoms, and a nitrogen atom. Again it would appear that the platinum is in the platinic state, but it must be remembered that the weight of previous chemical evidence has pointed to the platinum being in the bivalent state.

Acetylene compounds analogous to the ethylene complexes have not been obtained, and attempts lead only to brownish intractable substances. However, the substituted acetylene  $\text{CMe}_2(\text{OH})\cdot\text{C}\equiv\text{C}\cdot\text{CMe}_2(\text{OH})$  (:- Un) has yielded a compound  $[\text{PtUn py Cl}_2]$  similar to the corresponding *trans*-ethylene complex.<sup>24</sup>

*Aminopyridine Complexes.*—As might be expected from its stereochemistry, 2-aminopyridine (apy) does not form chelate compounds with platinous chloride,<sup>25, 26</sup> but the compounds formed  $[\text{apy}_2\text{PtCl}_2]$  and  $[\text{apy}_4\text{Pt}]\text{Cl}_2$  are more stable than the corresponding pyridine or ammonia derivatives. The former, obtained by direct action of 2-aminopyridine on potassium chloroplatinate, has been assigned a *cis*-configuration, which is to be expected, and to account for its greater stability, A. M. Rubinshtein<sup>27</sup> suggests that the co-ordination takes place through the tertiary nitrogen atom whilst the amino-hydrogen atoms take part in hydrogen-bond formation with the adjacent chlorine atoms. More highly substituted pyridines, e.g., 5-iodo-2-aminopyridine, react directly with potassium chloroplatinate to yield, in this case, *trans*- $[\text{iapy}_2\text{PtCl}_2]$ , probably because steric hindrance prevents formation of the *cis*-compound. The iodoaminopyridine is readily replaced by pyridine to yield *trans*- $[\text{py}_2\text{PtCl}_2]$ .<sup>28, 29</sup>

*Thiosulphate Complexes.*—Surprisingly little research into platinum thiosulphate complexes had been done until D. I. Ryabchikov<sup>30</sup> started a very thorough study of them in 1938 and found that the thiosulphate ion is co-ordinated very strongly to  $\text{Pt}^{\text{II}}$ . Previously, P. Shottländer<sup>31</sup> had obtained  $\text{Na}_6[\text{Pt}(\text{S}_2\text{O}_3)_4]\cdot 10\text{H}_2\text{O}$  by action of excess of sodium thiosulphate

<sup>21</sup> G. B. Bokiï and E. E. Baishteii, *Doklady Akad. Nauk. S.S.S.R.*, 1943, **38**, 323.

<sup>22</sup> G. B. Bokiï and E. E. Vainshtein, *Compt. rend. Acad. Sci. U.R.S.S.*, 1943, **38**, 307.

<sup>23</sup> G. B. Bokiï, N. I. Usikov, and G. L. Trusevich, *Bull. Acad. Sci. U.R.S.S., Classe sci. chim.*, 1942, 413.

<sup>24</sup> A. D. Hel'man, S. Bukhovetz, and E. Meilakh, *Compt. rend. Acad. Sci. U.R.S.S.*, 1945, **46**, 105.

<sup>25</sup> A. M. Rubinshtein, *ibid.*, 1938, **20**, 575.

<sup>26</sup> A. M. Rubinshtein, *Bull. Acad. Sci. U.R.S.S., Classe sci. chim.*, 1944, 42.

<sup>27</sup> *Compt. rend. Acad. Sci. U.R.S.S.*, 1944, **43**, 59.

<sup>28</sup> A. M. Rubinshtein, *Bull. Acad. Sci. U.R.S.S., Classe sci. chim.*, 1944, 216.

<sup>29</sup> A. M. Rubinshtein, *Compt. rend. Acad. Sci. U.R.S.S.*, 1944, **44**, 277.

<sup>30</sup> *Ibid.*, 1938, **18**, 39.

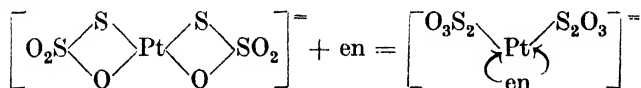
<sup>31</sup> *Annalen*, 1866, **140**, 200.

on potassium chloroplatinite, but the co-ordinating affinity of the thio-sulphate radical is such that the halogen atoms of the  $[\text{PtCl}_4]^-$  ion can be replaced two at a time by action of sodium thiosulphate in theoretical proportions,<sup>32</sup> yielding the ions  $[\text{Pt}(\text{S}_2\text{O}_3)\text{Cl}_2]^=$ ,  $[\text{Pt}(\text{S}_2\text{O}_3)_2]^=$ ,  $[\text{Pt}(\text{S}_2\text{O}_3)_3]^{4-}$ , and finally  $[\text{Pt}(\text{S}_2\text{O}_3)_4]^{6-}$ . These complexes are very stable; even hot hydrochloric acid fails to produce elementary sulphur or other appreciable change in them and the thiosulphate ion occupies either one or two co-ordination places.

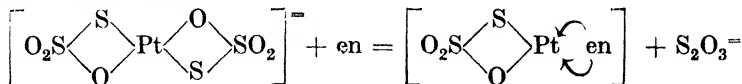
The extraordinary co-ordinating affinity of the thiosulphate ion is strikingly illustrated by the action of sodium thiosulphate on  $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$ .<sup>33</sup> Normally, the replacement of the ammonia molecules by acid radicals requires an excess of reagent and does not proceed easily, but thiosulphate in theoretical quantity reacts in hot solution with evolution of ammonia to produce  $[\text{Pt}(\text{NH}_3)_3\text{S}_2\text{O}_3]$  or *trans*- $\text{Na}_2[\text{Pt}(\text{NH}_3)_2(\text{S}_2\text{O}_3)_2]\cdot 6\text{H}_2\text{O}$  according to the proportions of the reagents, and excess of thiosulphate yields  $\text{Na}_2[\text{Pt}(\text{S}_2\text{O}_3)_4]$ . Even thiourea is completely evicted from  $[\text{Pt}\{\text{CS}(\text{NH}_2)_2\}_4]^{++}$  by excess of thiosulphate.

Particular interest attaches to the ion  $[\text{Pt}(\text{S}_2\text{O}_3)_2]^=$  which has been obtained in *cis*- and *trans*-forms,<sup>32</sup> an isomerism very common amongst the cationic and neutral platinous complexes but very rarely observed in anionic complexes. The two ions are produced together when the chloroplatinite and thiosulphate (1.8 mols.) react in aqueous solution, and are readily separated by the great difference in solubility of their potassium salts. Ethylenediamine reacts differently with the two salts, and on the basis that oxygen co-ordination places are attacked in preference to sulphur, the isomers have been orientated by the following reactions :<sup>34</sup>

*Soluble isomer.*



*Sparingly soluble isomer.*



Hence the soluble isomer is *cis*-, and the less soluble is *trans*-.

Ryabchikov also finds that the group *trans*- to the sulphur atom in the thiosulphate complexes is labilised in the same way as it is by thiourea. This fact strengthens his argument regarding the orientation of  $[\text{Pt}(\text{S}_2\text{O}_3)_2]^=$  and is well illustrated by comparison of the reaction between thiosulphate and *cis*- and *trans*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ ,<sup>35</sup> which he has suggested as useful to distinguish *cis*- and *trans*-isomers of platinum diammines.<sup>36</sup> Both isomers

<sup>32</sup> D. I. Ryabchikov, *Compt. rend. Acad. Sci. U.R.S.S.*, 1940, **27**, 349.

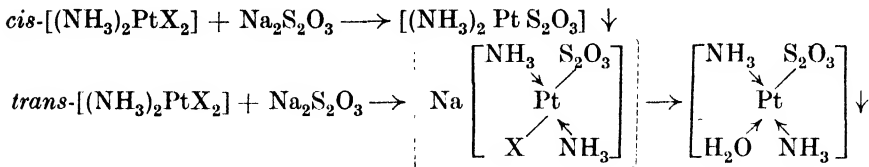
<sup>33</sup> *Idem, ibid.*, p. 690.

<sup>34</sup> *Idem, ibid.*, 1943, **41**, 208.

<sup>35</sup> *Idem, ibid.*, 1940, **28**, 231.

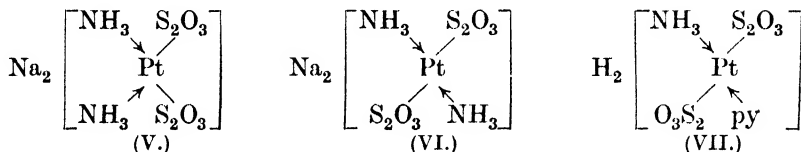
<sup>36</sup> *Idem, ibid.*, 1941, **32**, 344.

react with one molecule of thiosulphate to give sparingly soluble precipitates but these have different metal contents.



The labilising influence of the thiosulphate radical causes the X radical in the *trans*-position in the unstable intermediate to be replaced by a water molecule.

Two molecules of sodium thiosulphate substitute both acid radicals in both isomers, but the products (V) and (VI) differ markedly in their stability towards excess thiosulphate, for, whilst (V), having both ammonia molecules labilised by *trans*-thiosulphate radicals, reacts with any slight excess of



thiosulphate, yet (VI) is stable to 2–4 molecules excess of thiosulphate. Even the acid (VII), prepared from the barium salt by means of sulphuric acid, is stable in aqueous solution and is a strong acid.<sup>37</sup> Larger excess of thiosulphate replaces all the ammonia from both isomers.

The converse eviction of thiosulphate ions by amines is possible,<sup>38</sup> but even thiourea in hot aqueous solution can replace only three thiosulphate radicals from  $\text{K}_6[\text{Pt}(\text{S}_2\text{O}_3)_4]$  to give  $[\text{Pt}\{\text{CS}(\text{NH}_2)_2\}_3\text{S}_2\text{O}_3]$ . Ammonia replaces only two to yield *cis*- $\text{K}_2[(\text{NH}_3)_2\text{Pt}(\text{S}_2\text{O}_3)_2]$ , and ethylenediamine behaves similarly, whereas pyridine, presumably through the intermediate formation of *cis*- $\text{K}_2[\text{py}_2\text{Pt}(\text{S}_2\text{O}_3)_2]$ , removes two thiosulphate ions, but because of the labilising effect of the *trans*-thiosulphate ions, the pyridine is lost and the final product is  $\text{K}_2[\text{Pt}(\text{S}_2\text{O}_3)_2]$ ; *trans*- $\text{K}_2[\text{py}_2\text{Pt}(\text{S}_2\text{O}_3)_2]$  is, of course, quite stable.<sup>33</sup>

Thiosulphate complexes of quadrivalent platinum could not be obtained either directly<sup>39</sup> or by oxidation of the platinous complexes.<sup>40</sup> In the former case the platinum was reduced to the bivalent state by the thiosulphate, and in the latter the oxidation occurred in three stages, the first of which was oxidation of the thiosulphate with deposition of elementary sulphur. The platinum salt was then oxidised and finally the sulphur.

Palladium forms similar thiosulphate complexes,<sup>41</sup> but as would be

<sup>37</sup> D. I. Ryabchikov, *Compt. rend. Acad. Sci. U.R.S.S.*, 1940, **28**, 236.

<sup>38</sup> *Idem, ibid.*, 1943, **40**, 229.

<sup>39</sup> *Idem, ibid.*, 1944, **42**, 178

<sup>40</sup> *Idem, ibid.*, 1941, **33**, 233.

<sup>41</sup> D. I. Ryabchikov and A. P. Isakova, *Doklady Acad. Nauk. S.S.S.R.*, 1943, **41**,

expected no isomerism was observed, and the equimolecular reaction of thiosulphate and palladochloride produced  $\text{PdS}$  and  $\text{PdS}_2\text{O}_3$  but not  $\text{Na}_2[\text{Pd}(\text{S}_2\text{O}_3)\text{Cl}_2]$ , which is in keeping with the lower stability of palladium complexes usually observed.

*Hydroxylamine Complexes.*—The lower stability of palladium complexes is well illustrated by the reaction of halogen acids with  $[\text{Pt}(\text{NH}_2\cdot\text{OH})_4](\text{OH})_2$  and its palladium analogue described by Goremykin and his co-workers in their comparative study of these complexes.<sup>42, 43</sup>

Products from—

Acid.	$[\text{Pt}(\text{NH}_2\cdot\text{OH})_4](\text{OH})_2$ .	$[\text{Pd}(\text{NH}_2\cdot\text{OH})_4](\text{OH})_2$ .
HF	$[\text{Pt}(\text{NH}_2\cdot\text{OH})_4](\text{HF}_2)_2$	$[\text{Pd}(\text{NH}_2\cdot\text{OH})_4](\text{HF}_2)_2$
HCl	$[\text{Pt}(\text{NH}_2\cdot\text{OH})_4]\text{Cl}_2$	$[\text{Pd}(\text{NH}_2\cdot\text{OH})_4]\text{Cl}_2 + [\text{Pd}(\text{NH}_2\cdot\text{OH})_2\text{Cl}_2]$
HBr	$[\text{Pt}(\text{NH}_2\cdot\text{OH})_4]\text{Br}_2 + [\text{Pt}(\text{NH}_2\cdot\text{OH})_2\text{Br}_2]$	$[\text{Pd}(\text{NH}_2\cdot\text{OH})_2\text{Br}_2]$
HI	$[\text{Pt}(\text{NH}_2\cdot\text{OH})_4]\text{I}_2 + [\text{Pt}(\text{NH}_2\cdot\text{OH})_2\text{I}_2]$	$\text{PdI}_2$

Direct oxidation of the platinous hydroxylamine complexes by chlorine or bromine does not yield the platonic complexes, the hydroxylamine being oxidised in preference to the platinum,<sup>44</sup> but they have been prepared in a very interesting way.<sup>45, 46</sup>

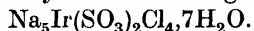
When a  $[\text{Pt}(\text{NH}_2\cdot\text{OH})_4]^{++}$  salt is heated on a water-bath with 20—48% hydrobromic acid the platinum is oxidised, presumably by the hydroxylamine liberated from the complex, and bright orange insoluble derivatives of  $\text{Pt}^{\text{IV}}$  can be isolated, *e.g.*,  $[\text{Pt}(\text{NH}_2\cdot\text{OH})_2\text{Br}_4]$ . Starting from mixed *cis*-tetramines, *e.g.*,  $[\text{Pt}(\text{NH}_2\cdot\text{OH})_2\text{py}_2]^{++}$ , mixed derivatives of type  $[\text{Pt}(\text{NH}_2\cdot\text{OH})\text{py}\text{Br}_4]$

are obtained.

Unlike the platinous hydroxylamine complexes, the platonic complexes decompose without explosion when they are heated, and also the hydroxylamine can be replaced by pyridine. In the latter reaction the liberated hydroxylamine reduces the platinum to the bivalent state again.

### *Iridium.*

*Sulphito-complexes.*—Lebedinsky and Gurin have made a study of chlorosulphitoiridites and aminosulphitoiridites in which iridium is trivalent. By heating sodium chloroiridite with an excess of sodium bisulphite three chlorosulphitoiridites are obtained according to the time of reaction.<sup>47</sup> If the heating is stopped when the olive-green solution has become light red, yellow  $\text{Na}_7\text{Ir}(\text{SO}_3)_4\text{Cl}_2\cdot 7\text{H}_2\text{O}$  crystallises out together with red



If the reaction is continued until the solution is dark red then only the red salt crystallises out and the yellow salt appears slowly from the

<sup>42</sup> V. I. Goremykin, *Compt. rend. Acad. Sci. U.R.S.S.*, 1938, **18**, 341.

<sup>43</sup> *Idem, ibid.*, 1941, **32**, 633.

<sup>44</sup> *Idem, Bull. Acad. Sci. U.R.S.S., Classe sci. chim.*, 1944, 185.

<sup>45</sup> V. I. Goremykin and K. A. Gladyshevskaya, *ibid.*, 1943, 108.

<sup>46</sup> *Idem, ibid.*, p. 338.

<sup>47</sup> V. V. Lebedinsky and M. M. Gurin, *Compt. rend. Acad. Sci. U.R.S.S.*, 1942, **36**, 22.

cold mother-liquor, whereas if the mother-liquor is evaporated by boiling, yellow  $\text{Na}_7\text{Ir}(\text{SO}_3)_4\text{Cl}_2 \cdot 5\text{H}_2\text{O}$  separates from the hot solution.<sup>48</sup> This pentahydrate retains one molecule of water up to  $170^\circ$ , whereas the heptahydrate loses all its water at  $100^\circ$ , but both salts yield  $\text{Na}_3\text{Ir}(\text{SO}_3)_3(\text{NH}_3)_3 \cdot 7\text{H}_2\text{O}$  with ammonia.

Conductivity measurements indicate that this ammine is only tri-ionic and it is considered that one of the sodium atoms is covalently linked in the complex.<sup>49</sup> By double decomposition with a zinc salt only two sodium atoms are replaced by zinc.

The parent salt  $\text{Na}_7\text{Ir}(\text{SO}_3)_4\text{Cl}_2 \cdot 5\text{H}_2\text{O}$  is also unusual in its reaction with dilute acid, which replaces two sodium atoms to yield a non-acidic crystalline substance  $\text{Na}_5\text{H}_2\text{Ir}(\text{SO}_3)_4\text{Cl}_2 \cdot 10\text{H}_2\text{O}$ . This has a very poor conductivity<sup>48</sup> and must have the hydrogen atoms and perhaps some sodium in the complex ion. The hydrogen atoms can be replaced by bases to produce again a neutral salt, and it seems probable that the above compounds are more complex than the simple formulæ would indicate.

$\text{Na}_5\text{Ir}(\text{SO}_3)_2\text{Cl}_4 \cdot 7\text{H}_2\text{O}$  also shows unexpected properties. One of the chlorine atoms is remarkably labile, conductivity measurements indicate dissociation into more than six ions at temperatures of over about  $30^\circ$ , and even in the cold rubidium chloride reacts to yield  $\text{NaRb}_3[\text{Ir}(\text{SO}_3)_2\text{Cl}_3] \cdot 6\text{H}_2\text{O}$ .<sup>47</sup>

The ammonium and potassium salts of the above chlorosulphitoiridites could not be obtained,<sup>48</sup> the tendency being to obtain salts of the ion  $[\text{Ir}(\text{SO}_3)_2\text{Cl}_3]^{4-}$  which seems to be identical with that originally described by C. Claus<sup>50</sup> in the salt  $\text{K}_4[\text{Ir}(\text{SO}_3)_2\text{Cl}_3] \cdot 6\text{H}_2\text{O}$ .

The prolonged action of large excess of ammonium bisulphite on ammonium chloroiridite did not replace all the chlorine atoms, but a new salt  $(\text{NH}_4)_5[\text{Ir}(\text{SO}_3)_3\text{Cl}_2]$ , which could readily be converted into the sodium or potassium salt by the alkali hydroxide, was formed.<sup>51</sup>

Although suggestions have been made regarding the configurations of most of the compounds studied, it was not possible to assign a configuration to any of them with certainty.

*Organic Arsine Complexes.*—Continuing their investigation of the compounds formed by the platinum metals in their lower valency states, F. P. Dwyer and R. S. Nyholm have prepared a number of complexes of iridium dichloride and trichloride with aryldialkyl- and diarylalkyl-arsines.<sup>52, 53</sup> These are consistent with six-fold co-ordination of iridium in both valency states. The simpler complexes  $[\text{IrCl}_2 \cdot 3\text{AsPh}_2\text{Me}]$  (VIII),  $[\text{IrCl}_2 \cdot 4\text{AsPh}_2\text{Me}]$  (IX), and  $[\text{IrCl}_3 \cdot 3\text{AsPh}_2\text{Me}]$  (X) are all less stable than the corresponding rhodium compounds<sup>54</sup> and smell of the free arsine. The halogen, on the other hand, is strongly bound and not readily removed even by silver nitrate. The compounds of type (IX) are not well defined

<sup>48</sup> V. V. Lobedinsky and M. M. Gurin, *Compt. rend. Acad. Sci. U.R.S.S.*, 1943, **38**, 128.

<sup>49</sup> *Idem*, *ibid.*, 1941, **33**, 241.

<sup>50</sup> *J. pr. Chem.*, 1847, **42**, 348.

<sup>51</sup> M. M. Gurin, *Compt. rend. Acad. Sci. U.R.S.S.*, 1944, **44**, 100.

<sup>52</sup> *J. Proc. Roy. Soc. N.S.W.*, 1944, **77**, 116.

<sup>53</sup> *Ibid.*, 1946, **79**, 121.

<sup>54</sup> *Ann. Reports*, 1944, **41**, 101.



and emit a strong odour of the free arsine; they are transformed into (VIII) by shaking with light petroleum. Compounds (VIII) and (IX) were the only complexes obtainable from iridium dihalides and diphenylmethylarsine. They were prepared by reduction of the tervalent iridium complexes in presence of different quantities of the arsine with hypophosphorous acid in acid aqueous-alcoholic solution. The complexes of type (VIII) were well defined and have been assigned a bridged structure (XI), but even in freezing benzene solution they are highly dissociated.

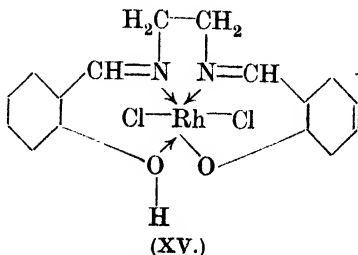
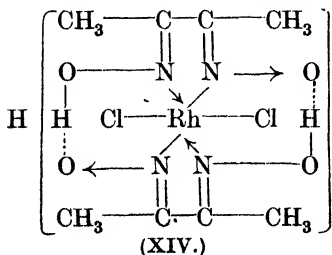
The simple complex (X) is obtained by direct action of the arsine on the halide  $\text{IrX}_3$  in weakly acid aqueous-alcoholic solution. In boiling strongly acid solution, however, a number of unexpected and interesting reactions occur.

Iridium trichloride yields a yellow, slightly soluble compound isomeric with (X), probably  $[\text{IrCl}_2, 4\text{AsPh}_2\text{Me}][\text{IrCl}_4, 2\text{AsPh}_2\text{Me}]$  (XII), and from the mother-liquor from which this compound has separated an acid  $\text{H}[\text{IrCl}_4, 2\text{AsPh}_2\text{Me}]$  (XIII) can be isolated. It is acid to litmus and gives pink ammonium and pyridinium salts but is insoluble in sodium hydroxide solution.

The analogous reaction in the bromine series yields the analogue of (XIII) but not of (XII); however, a complex containing bivalent iridium is precipitated, viz.,  $[\text{IrBr}_2, 2\text{AsPh}_2\text{Me}]$ . This reduction is unexpected and is probably facilitated by the low solubility of the complex formed. A similar reduction does not occur in the iodine series except with arylalkylarsines, but the iodides are quite soluble, and it appears that the instability of the iridium complexes is also important in helping this reduction which does not occur in the rhodium series although  $\text{Rh}^{\text{III}}$  is generally easier to reduce than  $\text{Ir}^{\text{III}}$ .

#### Rhodium.

*Dimethylglyoxime Complexes.*—In their search for square complexes of rhodium, F. P. Dwyer and R. S. Nyholm have prepared dimethylglyoxime complexes of bi- and ter-valent rhodium.<sup>55, 56</sup> With dimethylglyoxime, rhodic chloride readily yields a sparingly soluble substance, all the properties of which are consistent with the structure (XIV).



<sup>55</sup> *J. Proc. Roy. Soc. N.S.W.*, 1945, **78**, 266.

<sup>56</sup> *Idem, ibid.*, 1946, **79**, 126.

The complex is a strong acid of pronounced monobasic character. It forms stable soluble salts and the halogen atoms cannot be removed even by boiling silver nitrate or chelating acid groups. The silver salt is insoluble in water but soluble in dilute nitric acid. The chlorine atoms are almost certainly in the *trans*-position, particularly as the dimethylglyoxime can be reversibly replaced by ethyleneiminebis-salicylaldehyde to yield a complex (XV) which must have the nitrogen and oxygen atoms in one plane. This latter complex also yields a violet sodium salt which would indicate benzenoid-quinonoid resonance of the ion.

The rhodic complex  $\text{Rh}(\text{C}_4\text{H}_7\text{N}_2\text{O}_2)_3$  was ultimately prepared in poor yield as an insoluble powder from rhodic sulphate. It dissolved in hydrochloric acid to give a reddish solution, possibly of the *cis*-form of (XIV), which lightened in colour and finally deposited the stable *trans*-isomer (XIV).

Pure rhodous complexes were not isolated, although evidence for their formation by reduction of compound (XIV) with sodium formate was found.

Continuing his study of the polarographic reduction of the platinum metal complexes, J. B. Willis<sup>57</sup> finds that, of the metals ruthenium, osmium, iridium, palladium, and platinum, only palladium complexes give a satisfactory polarographic step. This corresponds to the reduction of  $\text{Pd}^{\text{II}}$  to Pd, and the half-wave potential of the ammino-complexes of palladium becomes more negative with increasing basic strength of the amine whilst the reduction also becomes less reversible.

Finally, attention should be directed to an excellent review of the stereochemistry of square complexes by D. P. Mellor.<sup>58</sup> J. C.

### 3. THE INORGANIC CHEMISTRY OF SOME METALLURGICAL PROCESSES.

The enforced development of special metallurgical processes during war years has necessarily involved new advances in, and applications of, fundamental inorganic chemistry, and several of these appear to merit review in these Reports. The topics selected for discussion are the extraction of magnesium (particularly from sea water), the production of highly electro-positive metals by thermal reduction processes, the extraction of alumina and aluminium from clay, and the extraction chemistry of beryllium and zirconium.

*Magnesium from Sea Water.*—The most successful of the commercial sea water processes is the Dow process, operated on a very large scale at Velasco, Texas.<sup>1</sup> Here the filtered sea water (containing about 0.13% of magnesium) is treated with a controlled excess of calcium hydroxide (prepared by slaking lime obtained by calcining oyster shells), and magnesium hydroxide is precipitated; by thickening the hydroxide is obtained as a

<sup>57</sup> J. Amer. Chem. Soc., 1945, **67**, 547.

<sup>58</sup> Chem. Reviews, 1943, **33**, 137.

<sup>1</sup> W. P. Schambra, Trans. Amer. Inst. Chem. Eng., 1945, **41**, 35; C. M. Shigley, Amer. Inst. Min. Met. Eng., Tech. Publ. No. 1845 (1945).

slurry containing 12% of  $\text{Mg}(\text{OH})_2$ . After filtration, the filter cake [25%  $\text{Mg}(\text{OH})_2$ ] is treated with hydrochloric acid solution containing a little sulphuric acid (to aid precipitation of calcium as sulphate), and the resulting crude 15% magnesium chloride solution is concentrated by submerged combustion of natural gas, a controlled gas-air mixture being burned under the liquid surface. This direct heating is necessary because calcium sulphate would cause serious scaling of any ordinary form of evaporator. After evaporative cooling under vacuum, a 35% magnesium chloride solution is obtained; a calculated quantity of magnesium sulphate, sufficient to precipitate the unwanted calcium, is added, and the solution allowed to stand. Filtration from precipitated sodium chloride and calcium sulphate then gives a magnesium chloride solution of high purity, from which a solid salt of the approximate composition  $\text{MgCl}_2 \cdot 1.5\text{H}_2\text{O}$  is obtained by a two-stage evaporation process. This salt is suitable for direct feed to the electrolytic magnesium cells, in which the electrolyte consists of molten magnesium, calcium, and sodium chlorides at 700–750°.² The chlorine evolved at the cell anodes is converted into hydrochloric acid (for re-use in the process) by reaction with steam and natural gas. The molten magnesium is ladled from the cells and cast into ingots of purity at least 99.9%.

An interesting variant of the Dow process uses calcined and slaked dolomite (comprising a mixture of magnesium and calcium hydroxides) instead of slaked lime in the initial treatment of the sea water; the magnesium content of the dolomite is then retained with the hydroxide precipitated from the sea water, and the process affords an economic means of utilising both sources of magnesium.³

The success of these processes is basically dependent on the very low solubility of magnesium hydroxide, which permits its precipitation from extremely dilute solutions of magnesium salts. Although the engineering problems involved in the treatment of large volumes of sea water are considerable, and each stage of the process requires careful control, both methods have been successfully applied.

*Magnesium from Dolomite and Silicate Minerals.*—The abundance of dolomite ( $\text{MgCO}_3 \cdot \text{CaCO}_3$ ) in nature immediately suggests that its use as a source of magnesium should be economic, but the difficulty of separating magnesium from large amounts of calcium is considerable. The use of dolomite in conjunction with sea water has been outlined above; another typical dolomite process has been described recently.⁴ The dolomite is first calcined and slaked with water to give a mixture of calcium and magnesium hydroxides, which is boiled with ammonium chloride solution; calcium then goes into solution as the chloride, whereas magnesium hydroxide remains substantially unaffected:  $\text{Mg}(\text{OH})_2 + \text{Ca}(\text{OH})_2 + 2\text{NH}_4\text{Cl} \longrightarrow \text{Mg}(\text{OH})_2 + \text{CaCl}_2 + 2\text{NH}_3 + 2\text{H}_2\text{O}$ . The magnesium hydroxide may be separated by

² R. M. Hunter, *Trans. Electrochem. Soc.*, 144, 86, Preprint 30, 343.

³ See P. L. Teed, *Bull. Inst. Min. Met.*, 1946, No. 479, 25.

⁴ J. M. Avery and R. F. Evans, *Amer. Inst. Min. Met. Eng.*, Tech. Publ. No. 1829 (1945).

thickening and filtration and converted into oxide by ignition, or the slurry from the previous stage may be treated directly with carbon dioxide to precipitate calcium carbonate and leave magnesium chloride in solution :  $\text{Mg}(\text{OH})_2 + \text{CaCl}_2 + \text{CO}_2 \longrightarrow \text{MgCl}_2 + \text{CaCO}_3 + \text{H}_2\text{O}$ . Purified magnesium chloride may then be obtained from the solution by methods similar to those used in the Dow process. Economic application of the process just described is ensured by linking it with the ammonia-soda process, so that the reaction of ammonium chloride with slaked dolomite calcine provides the necessary means of recycling ammonia gas.

Olivine,  $(\text{Mg}, \text{Fe})_2\text{SiO}_4$ , and other silicate minerals of magnesium are an attractive source of the metal in some localities. Such minerals are conveniently attacked by hydrochloric acid, which extracts magnesium and iron as chlorides and leaves the silica substantially insoluble; <sup>5</sup> the use of 20% acid at 90—110° ensures separation of silica in a form that settles well on standing. Impurities in the acid extract (mainly iron) are precipitated as hydrated oxides by adding the requisite quantity of magnesia, either as such or in the form of a sludge from electrolytic magnesium cells, containing magnesium chloride and oxide. Magnesium chloride of sufficient purity for cell-feed is obtained from the solution by evaporation.

*Electropositive Metals by Thermal Reduction.*—Until quite recently the difficulty of reducing oxides or salts of metals such as magnesium, calcium, and potassium has necessitated the production of these metals by electrolytic methods. Considerable use is now made of direct thermal reduction processes, particularly for magnesium, their industrial application having been promoted by development of plant operating under high vacuum.

The reduction of magnesium oxide by carbon at temperatures approaching 2000° has for some time been known to be possible; the use of this reaction ( $\text{MgO} + \text{C} \rightleftharpoons \text{Mg} + \text{CO}$ ) is hindered by its rapid reversal at somewhat lower temperatures, the magnesium vapour produced tending to react with carbon monoxide before it can be condensed. The equilibrium pressures of magnesium vapour and carbon monoxide are calculated to reach one atmosphere at 1851°, but they fall to less than 0.1 atmosphere at 1600°. <sup>6</sup> Recent success with the carbon reduction process has depended on very rapid "quenching" of the hot product gases with hydrogen, natural gas, or a spray of mineral oil. <sup>7</sup> This serves the double purpose of cooling the gases to a temperature at which the back-reaction occurs to a negligible extent, and of slowing down this reaction by extensive dilution of the reactants with inert gas. The magnesium, contaminated with oxide and free carbon, is recovered as a pyrophoric powder. In one typical application of this process <sup>8</sup> the magnesium oxide (prepared from dolomite and sea water) is compressed into pellets with petroleum coke, and the pellets are fed continuously into an electric-arc furnace lined with carbon blocks. As soon as the product gases

<sup>5</sup> E. C. Houston, *Amer. Inst. Min. Met. Eng.*, Tech. Publ. No. 1828 (1945).

<sup>6</sup> K. K. Kelley, see ref. (8).

<sup>7</sup> See P. L. Teed, *Bull. Inst. Min. Met.*, 1946, No. 479, 25.

<sup>8</sup> T. A. Duncan, *Amer. Inst. Min. Met. Eng.*, Tech. Publ. No. 1671 (1944).

leave the reaction zone they meet a cold blast of natural gas issuing from cooled jets mounted annularly round the exit pipe, and the average gas temperature quickly falls to about 250°. The condensed magnesium dust is collected in a large drum through which the gases pass and (mainly) in woollen bag filters; it is then collected, without exposure to air, made into a paste with asphaltic material, or into briquettes, and transferred to sublimation retorts. In these retorts, heated to 800°, the pressure is reduced to 0.2 mm. or less, and magnesium of high purity sublimes on to a cylindrical steel liner placed in the cooled head of each retort. After admission of hydrogen and cooling, the liners are removed, and the magnesium is stripped off.

Reducing agents other than carbon have been widely used in the thermal reduction of magnesia, and successful use of ferrosilicon, calcium carbide, or aluminium is reported. The ferrosilicon process<sup>9</sup> is notable for its simplicity, and for the fact that calcined dolomite may be used directly to supply part or all of the magnesium, the reaction being as follows:  $2\text{MgO} + \text{CaO} + \text{Si}$  (from ferrosilicon)  $\longrightarrow 2\text{Mg} + 2\text{CaO}, \text{SiO}_2$ . Use of this reaction at readily accessible temperatures depends on the maintenance of a high vacuum; in practice, briquettes of calcined dolomite and ferrosilicon, containing a little calcium fluoride, are charged into steel retorts, which are heated to about 1150° and pumped down to 0.05 mm. pressure. Magnesium condenses in the cooled head of each retort. Traces of alkali-metal salts in the charge give a small condensate of alkali metal, which may set fire to the magnesium when the retort is opened; this danger is minimised by condensing the more volatile alkali metal in the retort cap, which is quickly removed when air is admitted.

Calcium carbide and aluminium are used as reducers in a very similar manner,<sup>7</sup> the reactions involved being  $\text{MgO} + \text{CaC}_2 \longrightarrow \text{Mg} + \text{CaO} + 2\text{C}$ ;  $3\text{MgO} + 2\text{Al} \longrightarrow 3\text{Mg} + \text{Al}_2\text{O}_3$ . The use of small additions of calcium fluoride to the charge appears to be general in most of the processes described, although the mechanism by which it promotes the reaction is admittedly obscure.

It has been found that calcium can be produced from lime conveniently and economically in plant designed for the ferrosilicon reduction of magnesia,<sup>10</sup> if aluminium is employed as the reducer at about 1200°. The reaction is  $6\text{CaO} + 2\text{Al} \longrightarrow 3\text{Ca} + 3\text{CaO}, \text{Al}_2\text{O}_3$ . Since other alkaline-earth and alkali metals present in the charge distil with the calcium, the use of high-purity lime is important.

The regular production of potassium metal by thermal reduction is reported from Germany.<sup>11</sup> Potassium fluoride is reduced at 1000–1150° with calcium carbide ( $2\text{KF} + \text{CaC}_2 \longrightarrow 2\text{K} + \text{CaF}_2 + 2\text{C}$ ) or silicon, lime being added in the latter case to combine with silica formed in the reaction

<sup>9</sup> L. M. Pidgeon, *Canad. Mining and Met. Bull.*, 1944, No. 381.

<sup>10</sup> P. H. Staub, *Chem. and Met. Eng.*, 1945, 52, No. 8, 94; C. C. Loomis, *Trans. Electrochem. Soc.*, 1946, 89, Preprint 9, 119.

<sup>11</sup> *F.I.A.T. Final Report*, No. 695.

$(4\text{KF} + \text{Si} + 4\text{CaO} \longrightarrow 4\text{K} + 2\text{CaF}_2 + 2\text{CaO}, \text{SiO}_2)$ . The process is carried out in steel retorts; the metal distils out of the reaction mixture, and is condensed and collected under petroleum. Part of the potassium fluoride may be substituted by potassium carbonate  $[2\text{K}_2\text{CO}_3 + 3\text{Si} + 6\text{CaO} \longrightarrow 4\text{K} + 2\text{C} + 3(2\text{CaO}, \text{SiO}_2)]$  or silicate  $[2\text{K}_2\text{SiO}_3 + \text{Si} + 6\text{CaO} \longrightarrow 4\text{K} + 3(2\text{CaO}, \text{SiO}_2)]$  without appreciable loss of yield. All the reactants must be thoroughly dried; explosions are said to occur if moisture is present when potassium carbonate is used in the reaction.

*Aluminium from Clay and High-silica Bauxite.*—The extraction of alumina or aluminium metal from clay, its most abundant and accessible natural source, has been investigated over a long period, and a voluminous literature of the subject exists.<sup>12</sup> The objectionable impurities likely to occur in alumina derived from clay are silica and iron, and each of the two main types of process generally proposed deals effectively with one only of these impurities; "acid" extraction methods applied to clay lead to rapid separation from silica, elimination of iron being difficult, whereas "alkali" processes, generally depending on an extraction of soluble sodium aluminate by water, cause difficulty with removal of silica.

A recent careful study<sup>13</sup> of a sulphuric acid process for treatment of clay clearly indicates the difficulties associated with this method. The calcined clay is leached with sulphuric acid, and iron is precipitated from the resulting aluminium sulphate solution by treatment with manganous sulphate and ozone. After partial concentration, a clay residue is added to promote precipitation of silica. The purified solution is evaporated (by submerged combustion), and the aluminium sulphate dehydrated and calcined to alumina; the sulphur oxides evolved in the calcination are recovered as sulphuric acid for use in the first stage of the process.

An alternative to the leaching of clay with sulphuric acid is roasting with ammonium sulphate<sup>14</sup> (or in some similar processes, ammonium hydrogen sulphate<sup>15</sup>). Leaching of the product with water gives a solution from which ammonium alum may be crystallised; this is converted into hydrated alumina by treatment with ammonia evolved in the roasting stage. Ammonium sulphate can be recovered and re-cycled through the process.

An interesting recent "acid" process<sup>16</sup> uses a combination of sulphuric and sulphurous acid leaching of clay, alumina being recovered by precipitation of a basic aluminium sulphate from the leach liquor. The basic salt is afterwards dissolved in sodium hydroxide solution (giving sodium aluminate), and hydrated alumina is precipitated by controlled addition of

<sup>12</sup> See a recent bibliography by R. J. Woody, *Washington State Coll., Electromet. Res. Lab.*, Bull. E-1 (1943).

<sup>13</sup> J. H. Walthall, P. Miller, and M. M. Striplin, jun., *Trans. Amer. Inst. Chem. Eng.*, 1945, **41**, 55.

<sup>14</sup> H. W. St. Clair, S. F. Ravitz, A. T. Sweet, and C. E. Plummer, *ibid.*, 1944, **159**, 255.

<sup>15</sup> Anon., *Mining World*, 1945, **7**, No. 10, 22.

<sup>16</sup> O. Redlich, C. C. March, M. F. Adams, F. H. Sharp, E. K. Holt, and J. E. Taylor, *Ind. Eng. Chem.*, 1946, **38**, 1181.

sulphuric acid. The sodium sulphate solution remaining is electrolysed to give sulphuric acid and sodium hydroxide solutions for use in earlier steps of the process.

The most useful of the "alkali processes" for treatment of clay appears to be the "lime-soda sinter" process, long known but largely investigated in the United States during the War.<sup>17</sup> If clay is sintered at a moderately high temperature with controlled quantities of sodium carbonate and lime, its aluminium content is converted into sodium aluminate ( $\text{NaAlO}_2$ ), and its silica into an insoluble calcium silicate, probably  $2\text{CaO} \cdot \text{SiO}_2$ . In theory, extraction of the sinter with water should give a sodium aluminate solution substantially free from silica, from which hydrated alumina could be precipitated by "seeding" with the hydrate, or by treatment with carbon dioxide; in practice, however, the extract is found to contain appreciable amounts of silica, at least part of which is precipitated with the alumina, and the product requires re-processing by the usual Bayer procedure before it can be used for electrolytic production of aluminium.

The lime-soda sinter process has been more usefully applied to low-grade bauxites containing much silica.<sup>18</sup> In the usual Bayer process bauxite is treated with hot sodium hydroxide solution under pressure, to give a silica-free sodium aluminate solution from which a pure alumina hydrate is precipitated. If the bauxite contains much silica, uneconomic amounts of both aluminium and sodium are retained in the Bayer treatment residue ("red mud") as an insoluble sodium aluminium silicate. Recovery of the sodium and aluminium may be effected by sintering the red mud from a high-silica bauxite with sodium carbonate and lime in suitable proportions, and leaching the product with water. The extract contains sodium aluminate with a little silica, but if the solution is added to the alkali liquor used in a succeeding Bayer treatment, this silica is precipitated during the pressure digestion. This ingenious addition to the well-established alumina process has extended its useful application to poor-quality ores.

"Lime-sinter" processes have also been investigated recently.<sup>19</sup> In these, the clay is sintered at a high temperature with lime, and the silica and alumina contents are converted into calcium silicates and aluminates. On treating the sinter with sodium carbonate solution, the silicates are unchanged, but the aluminates undergo double decomposition, calcium carbonate remaining in the residue and sodium aluminate being formed in solution. Alumina can be precipitated from the aluminate extract by the usual methods, the residual alkali being returned to the process as sodium carbonate.

In a somewhat similar German process,<sup>20</sup> clay has been sintered with coke

<sup>17</sup> *Univ. Kansas Publ., Kansas State Geol. Survey Bull.*, 1943, **47**, 114.

<sup>18</sup> *Chem. and Met. Eng.*, 1945, **52**, No. 1, 106; J. D. Edwards, *Amer. Inst. Min. Met. Eng.*, Tech. Publ., No. 1833 (1945).

<sup>19</sup> R. L. Copson, J. H. Walthall, and T. P. Hignett, *Trans. Amer. Inst. Min. Met. Eng.*, 1944, **159**, 241; F. R. Archibald and C. F. Jackson, *Amer. Inst. Min. Eng. Tech. Publ.*, No. 1706 (1944).

<sup>20</sup> *C.I.O.S. Report*, No. XXXII—21.

and anhydrite ( $\text{CaSO}_4$ ) to give a product apparently consisting of calcium aluminate and silicate; on treatment with sodium carbonate solution this affords sodium aluminate solution and a residue (calcium carbonate, silicate, etc.) which can be recalcined to a cement of good quality.

Some clay processes of a quite different type have also been investigated in Germany.<sup>20</sup> In these the first step is a direct reduction of the alumina-silica ore with carbon in an electric-arc furnace, giving an alloy ("silumin") containing about 60% of aluminium, with silicon and a little iron. Aluminium is then extracted from the finely divided alloy by treatment with mercury or molten magnesium. At about 600° and 22 atmospheres pressure mercury gives a solution containing nearly 40% of aluminium; on cooling, almost all the aluminium is precipitated and can be removed by a filtration process, adhering mercury being removed subsequently by vacuum distillation. If magnesium is used for the extraction it is recovered directly from the alloy by vacuum distillation. The silicon-iron residue may in either case be used for thermal reduction of magnesia, an economic advantage of the process. Other methods suggested for the processing of silumin are extraction with molten lead and volatilisation of aluminium subfluoride.<sup>21</sup>

*Beryllium.*—The extraction of beryllium and its compounds is complicated by the difficulty of attacking beryl ( $3\text{BeO}, \text{Al}_2\text{O}_3, 6\text{SiO}_2$ ), the only ore available in large quantities, and by the somewhat difficult separation of beryllium from the accompanying aluminium. The simplest method of attack is to fuse the beryl in a carbon-lined electric arc furnace at not less than 1500—1600° and quench the melt in cold water; the resulting vitreous mass, after crushing, reacts readily with concentrated sulphuric acid, the beryllium and aluminium being converted into partly hydrated sulphates.<sup>22</sup> The mass of sulphates, containing insoluble silica, is leached with water, and ammonium sulphate is added to the solution to precipitate the bulk of the aluminium as ammonium alum,  $(\text{NH}_4)_2\text{Al}(\text{SO}_4)_2, 12\text{H}_2\text{O}$ , which has a very low solubility in beryllium sulphate solution containing ammonium sulphate. Crude hydrated beryllium sulphate is crystallised from the filtrate, and subsequently purified by a recrystallisation procedure. High-grade beryllium oxide is obtained from the sulphate by ignition at 1350°.

A process used in Germany<sup>23</sup> is similar in many respects. The beryl is fused with calcium oxide at about 1500°, and the quenched melt "sulphated" by treatment with sulphuric acid; aluminium is removed as ammonium alum, as before, and the crude solution of beryllium sulphate is freed from iron (present initially as ferrous salt) by addition of hydrogen peroxide and calcium carbonate, which precipitate hydrated ferric oxide. On passing gaseous ammonia into the resulting beryllium sulphate solution, the hydroxide,  $\text{Be}(\text{OH})_2$ , is precipitated; this is finally ignited to the oxide at about 1000°.

Attack of beryl by fusion or sintering with fluoride or complex fluoride,

<sup>21</sup> C. B. Willmore, U.S.P. 2,184,705.

<sup>22</sup> B. R. F. Kjellgren, *Trans. Electrochem. Soc.*, 1946, **89**, Preprint 5, 83.

<sup>23</sup> *B.I.O.S. Final Report*, No. 158; *F.I.A.T. Final Report*, No. 522. p. 45.



originally a favourite method,<sup>24</sup> still persists in recent processes; the aim of the older fluoride methods of attack was usually to convert beryllium into soluble sodium beryllium fluoride,  $\text{Na}_2\text{BeF}_4$ , and aluminium into insoluble cryolite,  $\text{Na}_3\text{AlF}_6$ , so that a beryllium salt of moderate purity could be leached directly from the reaction product. Modern variants of the fluoride process are designed to extract the beryllium as a soluble fluoride and leave the aluminium oxide and silica from the ore substantially unattacked. In an Italian process<sup>25</sup> the beryl is sintered at about  $800^\circ$  with sufficient sodium hydrogen fluoride,  $\text{NaHF}_2$ , to convert all the beryllium present into a sodium beryllium fluoride, presumed (probably wrongly) to be  $3\text{NaF} \cdot 2\text{BeF}_2$ , which can be extracted with water from the residue of oxides. A more novel process<sup>26</sup> uses sodium ferric fluoride,  $\text{Na}_3\text{FeF}_6$ , as the attacking reagent; this reacts preferentially with beryllium oxide ( $3\text{BeO} + 2\text{Na}_3\text{FeF}_6 \longrightarrow 3\text{Na}_2\text{BeF}_4 + \text{Fe}_2\text{O}_3$ ) and leaves alumina, silica, and iron oxide (impurity) unattacked. The sodium beryllium fluoride solution from either method of attack is treated with alkali to precipitate beryllium hydroxide, preferably by adding an excess of sodium hydroxide to redissolve the initial precipitate, and then pouring a further quantity of sodium beryllium fluoride solution into the hot liquid; this procedure gives a granular, crystalline hydroxide which is convenient to filter off.<sup>26</sup> The filtrate contains sodium fluoride, and the economic use of the process demands recovery of its fluoride content; this is conveniently effected by adding ferric sulphate to precipitate sodium ferric fluoride [ $12\text{NaF} + \text{Fe}_2(\text{SO}_4)_3 \longrightarrow 2\text{Na}_3\text{FeF}_6 + 3\text{Na}_2\text{SO}_4$ ], which may be re-used in the attack of the ore.<sup>26</sup> The sodium ferric fluoride mode of attack is stated to be applicable with advantage to a new low-grade beryllium ore comprising magnetite with small quantities of helvite, a beryllium iron aluminium silicate. Processes of this type, in which the desired ore constituent is selectively attacked without consumption of reagents by unwanted material, are of special value in the utilisation of low-grade mineral deposits.

The isolation of beryllium metal was formerly effected by electrolysis of a fluoride melt, usually containing alkali or alkaline-earth fluoride with beryllium fluoride or oxyfluoride. This method required the use of relatively high electrolyte temperatures, and highly toxic fluorine gases evolved at the anodes were troublesome. Preference is now given to electrolysis of a melt of sodium and beryllium chlorides at about  $350^\circ$ .<sup>23</sup> Satisfactory application of this process depends on a supply of pure anhydrous beryllium chloride, now prepared by chlorinating briquettes of beryllium oxide and carbon at  $700\text{--}800^\circ$  ( $\text{BeO} + \text{C} + \text{Cl}_2 \longrightarrow \text{BeCl}_2 + \text{CO}$ ); the beryllium chloride sublimes out of the furnace, and is subsequently purified by fractional sublimation in a current of hydrogen.

Beryllium powder has been satisfactorily prepared from the chloride by reduction by sodium vapour at reduced pressure.<sup>27</sup> Beryllium fluoride may

<sup>24</sup> See, e.g., R. Rimbach and A. J. Michel, "Beryllium", New York, 1932.

<sup>25</sup> F.I.A.T. *Final Report*, No. 522, p. 62.

<sup>26</sup> H. C. Kawecki, *Trans. Electrochem. Soc.*, 1946, **89**, Preprint 11, 133.

<sup>27</sup> J. M. Tien, *ibid.*, Preprint 19, 223.

also be reduced with magnesium to give a pure metal.<sup>28</sup> Alloys of beryllium (particularly with copper or nickel) can be prepared by reducing beryllium oxide with carbon in presence of the free alloying metal,<sup>22, 28</sup> but the beryllium content obtainable in the alloy is limited; beryllium fluoride may similarly be reduced with magnesium in presence of base metal to give an alloy.<sup>28</sup>

*Zirconium.*—Considerable interest in zirconium, particularly in the metal in its ductile state,<sup>29</sup> has been evident recently, and the publication of a review<sup>30</sup> of much scattered information is timely.

In a typical recent process,<sup>31</sup> zircon sand (mainly  $\text{ZrSiO}_4$ ) is heated with carbon in an electric resistor furnace at about  $2000^\circ$ , and a mixture of zirconium and silicon carbides is formed; some of the silicon is said to be volatilised from the charge as the monoxide,  $\text{SiO}$ . The mixture of carbides is heated in chlorine, and the tetrachlorides of zirconium and silicon are formed in a strongly exothermic reaction; the zirconium tetrachloride can be fractionally condensed from the mixture in a vessel held above  $80^\circ$ , the condensate containing only 0.05–0.3% of silicon, with up to 0.5% of iron and other impurities. The chloride is purified by sublimation in hydrogen, which reduces ferric chloride to the relatively non-volatile ferrous chloride; the sublimate contains only 0.05% of iron. The purified zirconium tetrachloride is reduced with magnesium in a special furnace so designed that reaction occurs between the tetrachloride vapour and molten magnesium, a helium atmosphere in the furnace ensuring exclusion of air. The mixture of zirconium metal, magnesium chloride, and excess of magnesium produced in the reaction is vacuum-distilled at up to  $900^\circ$  in a second furnace; the residue of zirconium remaining may still contain up to 1% of magnesium chloride, magnesium, and hydrogen, which are removed by heating the metal in a vacuum induction furnace at  $1500^\circ$ . Finally, the zirconium is melted down into small ingots in an arc furnace in an atmosphere of helium at low pressure. The product is ductile and can readily be rolled into sheet.

Another method of reducing zirconium tetrachloride with magnesium has been described.<sup>32</sup>

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<sup>28</sup> W. J. Kroll, *U.S. Bur. Mines, Inf. Circ. No. 7326* (1945).

<sup>29</sup> See, for example, D. B. Alnutt and C. L. Scheer, *Trans. Electrochem. Soc.*, 1945, 88, Preprint 30, 357.

<sup>30</sup> W. J. Kroll and A. W. Schlechten, *U.S. Bur. Mines, Inf. Circ. No. 7341* (1946).

<sup>31</sup> W. J. Kroll, A. W. Schlechten, and L. A. Yerkes, *Trans. Electrochem. Soc.*, 1946, 89, Preprint 29, 365.

<sup>32</sup> H. von Zeppelin, *Metall u. Erz*, 1943, 40, 252.

# ORGANIC CHEMISTRY.

## 1. INTRODUCTION.

THE subjects selected for review in this section include the hydrogen bond, stereochemistry, carbohydrates, steroids, and a selection of heterocyclic compounds containing nitrogen.

A review is given of those methods of detection of the hydrogen bond which have proved of greatest use in the elucidation of organic structures. Emphasis is laid on the physical conditions of the methods by which assessment of structure is made, and whether these conditions are likely to preserve a hydrogen-bond structure. Methods based on the measurement of inter-atomic distances by means of both *X*-ray diffraction and electron diffraction are discussed, examples of the former being provided by phthalocyanine, melamine, and hyperol, and of the latter by hydrofluoric acid and carboxylic acids. Various indirect or comparative methods are also discussed, such as those based on infra-red absorption, volatility, solubility and related properties, and molecular weight determinations. Reference is made to the solute-solvent interaction revealed by the work of C. S. Marvel and his collaborators, and to methods for the detection of molecular association and internal hydrogen bonds (chelate rings). A review is included of the chemical differences between compounds possessing a hydrogen bond structure and others closely related to them, *e.g.*, *o*-hydroxyazo-compounds, the benzil monoximes; of methods of alternative syntheses leading to a single individual compound, *e.g.*,  $\beta$ -diketones, methylnaphthazarin, quinhydrone, formazyl compounds; and of the effect of chelation in stabilising certain structures and in favouring certain reactions.

The chief examples (mainly organic) of compounds exhibiting hydrogen bonds of the following types are considered: F-H-F, F-H-N, F-H-O, O-H-O, N-H-O, N-H-N, N-H-S, O-H-S. Some emphasis is laid on the important part played by the hydrogen bond in deciding crystal structure and other important physical properties, *e.g.*, density of ice, layer cleavage of anhydrous oxalic acid, configuration of proteins. The evidence for the engagement of the CH group in hydrogen-bond formation is considered in some detail (*i.e.*, C-H-O, C-H-N), and a short account of hydrogen bonds involving other elements (S, Cl, Br) is given. The close connection between hydrogen-bond association and tautomerism ("mesohydric tautomerism") is given brief mention.

An absolute asymmetric synthesis of ethyl *d*-tartrate from ethyl fumarate has been reported. Mixtures of diastereoisomeric esters have been separated by high-efficiency fractional distillation. The sulphur-oxygen bond in sulphoxides, formerly supposed to be a co-ordinate bond, is shown to be a double bond, so that the figure corresponding with the sulphur valencies is a trigonal bipyramid and not a "tetrahedron". Interesting stereochemical studies have been made of heterocyclic compounds of sulphur, selenium, and

tellurium, and very complete investigations of the optical activity of heterocyclic and *spirocyclic* compounds of arsenic have been described. A base, which owes its molecular dissymmetry to the presence of two ring nitrogen atoms with stable "tetrahedral" configurations, has been resolved into *d*- and *l*-forms. A beginning has been made in the direction of discussing quantitatively steric effects in replacement reactions, and continued successful use has been made of optical activity for the study of molecular rearrangements.

In view of the growing biological importance of the inositols and their derivatives, the opportunity has been taken to review the developments in this field, and an account has been given of the rarer methylpentose sugars and their deoxy-derivatives, which are found combined in the cardiac glycosides. The use of chromatography to separate qualitatively and quantitatively mixtures of sugars and their derivatives is discussed; the method has been developed on the micro-scale for the separation of methylated sugars and their glycosides. The section on the oxidation of  $\alpha$ -glycol groups (L. N. Owen, *Ann. Reports*, 1943, **40**, 115) with periodic acid and lead tetra-acetate has been continued. Sugar derivatives containing one or more anhydro-rings of the septano-, pyrano-, furano-, and ethylene oxide types have been prepared and their structures elucidated. The Walden inversion which occurs when an oxide ring is opened has been used to prove the configuration of the amino-group in chondrosamine and to prepare derivatives of the less accessible sugars.

A summary is given of the more recent constitutional work on the structure of the polysaccharides. A noteworthy advance has been made with the enzymatic synthesis of amylopectin, and the degradative action of  $\beta$ - and other amylases on this polysaccharide has received considerable attention. The polysaccharides from gum tragacanth, the  $\epsilon$ -galactan of the larch, and damson tree gum have received a detailed examination. Gum tragacanth has been shown to be a mixture; the  $\epsilon$ -galactan is also considered to be a mixture by some authors; damson tree gum appears to be homogeneous. All these polysaccharides are of the ramified type and contain a variety of sugars.

Stereochemical developments during the last eight years have confirmed the general picture of the steroid nucleus given by Ruzicka in 1933, and evidence has been adduced to show that the various possible geometrical modifications arising from chair-boat transformations of rings A and B make no contribution to the stereochemistry of the steroid nucleus. Methods available for determination of the orientation of nuclear substituents are summarised, and, in a review of the various nuclear positions, examples of their use are given. There is still no strict proof available to show that the hydroxyl group in cholesterol and cholestanol is ( $\beta$ )-orientated, but on the basis of this assumption it has been possible to establish the configuration at C<sub>3</sub> of a whole series of derivatives of androstane, androst-5-ene, and their homologues. Attention is drawn to the fact that the configuration is known at C<sub>7</sub> in the bile-acid series but not in the sterol series; also that it seems probable that the previous arbitrary allocation of configuration at C<sub>7</sub> in the

sterol series must be reversed. The proof given by Reichstein that the formerly accepted structure of deoxycholic acid is incorrect in regard to configuration at C<sub>12</sub> and C<sub>17</sub> has far-reaching repercussions; the same conclusion, namely that the hydroxyl group at C<sub>12</sub> and the side chain at C<sub>17</sub> are respectively ( $\alpha$ )- and ( $\beta$ )-orientated, has been reached independently and on quite different grounds by groups of workers in America. It follows, *inter alia*, that, as originally suggested by Reichstein and Shoppee, the C<sub>11</sub>-hydroxyl group characteristic of the natural adreno-cortical steroids has the ( $\beta$ )-configuration, and that the C<sub>17</sub>-side chain in cholesterol, progesterone, corticosterone, the steroid sapogenins, and the cardiac aglycones is ( $\beta$ )-orientated. A further consequence is that the *Digitalis* heart poisons must contain a *cis*-C/D-ring union, a view now accepted by Ruzicka, Plattner, and their co-workers who, by the synthesis of steroids with hydroxyl groups at C<sub>5</sub> and C<sub>14</sub>, are preparing the way to the synthetic production of these compounds.

In the field of reduced heterocyclic rings recent examples are discussed of the synthesis of piperidines and piperidones by (i) the reductive cyclisation of  $\gamma$ -cyano-esters, (ii) the Eisleb double alkylation method (of a reactive methylene group by di- $\beta$ -chloroethylalkylamines), and (iii) the Dieckmann-like cyclisation of di- $\beta$ -carbethoxyalkylamines. Attention is drawn to the preparative advantages arising from the use of *N*-benzoylated and -nitrosated derivatives as intermediates, and some new reactions of certain piperidines and piperidones are mentioned. Recent synthetic work in the reduced bicyclic field (*cycloalkano*-pyrrolidines, -piperidines, and -thiazoles, and *bicycloaza*-alkanes) is briefly reviewed, and the stereochemistry of  $\beta$ -biotin and its derivatives is summarised. A section on indoles deals with recent critical studies of the Bischler synthesis from arylamines and  $\alpha$ -halogeno-ketones, with improved preparative routes to tryptophan and indole-3-aldehyde, with the synthesis of 7-azaindoles, and with the chemistry of gliotoxin, the naturally-occurring antibiotic. Recent developments in the quinoline field are reviewed chiefly from the aspect of preparative improvements, particularly as applied to the synthesis of 4-hydroxyquinolines, a large number of which have been prepared by condensation of arylamines with ethyl ethoxymethylenemalonate followed by cyclisation or by variations of this method. Brief mention is made of progress in the chemistry of cinnoline derivatives. A comprehensive statement is given of the chemistry of the pterins, compounds which contribute to the pigmentation of the wings of insects and which, until recently, were regarded as of academic interest only. The chemistry of this group, which is related to the purines, has lately been greatly clarified and interesting and important biological properties have been revealed. This section includes a description of the synthesis and proof of structure of the antianæmic "Liver *L. casei* factor" (vitamin B<sub>6</sub>) and of its relationship to other active compounds, which are pterin derivatives, and with a general discussion of the vitamin B<sub>6</sub> problem.

W. B.

D. H. H.

## 2. THE HYDROGEN BOND.

The fact that hydrogen can sometimes link two other atoms together is now beyond all question, but the mechanism of this virtual bivalency still remains obscure. The resonance mechanism suggested by N. V. Sidgwick<sup>1</sup> is by no means universally accepted, and an electrostatic union, largely the result of the hydrogen atom being a bare proton with a minimum of enveloping electrons, has strong support.<sup>2</sup> The latter view is in harmony with the fact that the atoms linked by hydrogen bonds are confined almost entirely to the electronegative elements of small atomic radius (*i.e.*, N, O, F), and that hydrogen bonds between atoms other than these are of the weakest kind. As between these two views there is good evidence for believing that resonance, if it contributes at all to the structure of the hydrogen bond, does so to a very inconsiderable extent.<sup>3</sup>

*Methods of Detecting the Hydrogen Bond.*

It is important in this connexion to distinguish between hydrogen bonds formed intramolecularly (by chelate rings) and those formed between molecules. The former will usually lead to a unimolecular condition, the latter to molecular association, and the resultant physical properties of the substance concerned will be largely influenced by these alternatives. It is evident, too, that the physical conditions under which assessment of structure is made will determine whether or not a substance will preserve its hydrogen-bond structure; for example, a hydrogen-bond structure present in the solid or liquid state may well be destroyed in dilute solution or in the vapour state. Moreover, intermolecular hydrogen bonds are usually more sensitive than are intramolecular to the stresses imposed by vaporisation or dissolution.

The following is a brief summary of the chief methods in general use for the detection of hydrogen bonds.

*I. Interatomic Distance Methods.*—These depend on the measurement of the distance between the atoms linked by the hydrogen bond, and are based on the assumption that any approach of two such atoms to a distance significantly less than about 3.4 Å. indicates a chemical link between them. The strengths of such bonds are in the inverse order of these interatomic distances.

(a) *X-Ray diffraction.\** Applied mainly to crystals, which are most likely to favour a maximum display of hydrogen bonds, this method has yielded more information than any other about hydrogen-bond structures. It is, however, confined at present to relatively simple compounds (*e.g.*, inorganic salts), and becomes progressively more difficult to apply as

<sup>1</sup> *Ann. Reports*, 1933, **30**, 112; 1934, **31**, 40; "Organic Chemistry of Nitrogen", Oxford University Press, 1937, xvii.

<sup>2</sup> L. Pauling, "The Nature of the Chemical Bond", Cornell, 1940, p. 286.

<sup>3</sup> Mansel Davies, this vol., p. 29.

\* The scope of diffraction methods as a guide to molecular structure is reviewed by J. M. Robertson, Tilden Lecture, *J.*, 1945, 249.

molecular complexity increases. It is not surprising, therefore, that its application to organic hydrogen-bond structures has been limited to simple examples, although phthalocyanine,<sup>4</sup> melamine,<sup>5</sup> and hyperol<sup>6</sup> are notable exceptions. The method gives an accurate measure of the A-H-B distance, where A and B may be in the same or in different molecules, and, though it does not locate the hydrogen atom within this distance, it indicates with some certainty the presence or absence of the hydrogen bond. For example, the presence of the O-H-O bond in sodium hydrogen carbonate,<sup>7</sup> potassium dihydrogen phosphate<sup>8</sup> and arsenate,<sup>9</sup> and ammonium periodate,  $(\text{NH}_4)_2\text{H}_3\text{IO}_6$ ,<sup>10</sup> and its absence in ammonium hypophosphite,  $\text{NH}_4\text{H}_2\text{PO}_2$ ,<sup>11</sup> supports the chemical evidence that the former are true acid salts, whereas the latter is not.

(b) *Electron diffraction*.\* Owing to the fact that this method is usually applied to vapours at low pressures, it is unlikely to reveal intermolecular hydrogen bonds. It is for this reason that the structures of hydrogen peroxide<sup>12</sup> and of hydrazoic acid<sup>13</sup> determined by this method give no indication of hydrogen bonding, although the physical properties of the pure substances clearly point to molecular association by hydrogen bonds.<sup>14</sup> On the other hand, the F-H-F bond in hydrogen fluoride is sufficiently powerful to persist in the vapour, which is found<sup>15</sup> to consist of zigzag polymers having a F-H-F distance of 2.55 Å., the F-F-F angle being about 140°. This agrees well with the structure of solid hydrogen fluoride determined<sup>16</sup> by the X-ray method, and seems incompatible with previous cyclic structures. The method has also been applied to the simpler carbocyclic acids,<sup>17</sup> the dimeric structure of which survives vaporisation.

II. *Indirect (or Comparative) Methods*.—These depend very largely on the fact that the engagement of a group in hydrogen-bond formation modifies the physical, and to a lesser extent the chemical, properties of the group involved. They consist of a comparison of properties (many of which may be capable of numerical expression) displayed by substances which may possess a hydrogen-bond structure, with those of closely related

<sup>4</sup> J. M. Robertson, *J.*, 1936, 1195.

<sup>5</sup> (Miss) I. E. Knaggs and (Mrs.) K. Lonsdale, *Proc. Roy. Soc.*, 1940, A, **177**, 140; E. W. Hughes, *J. Amer. Chem. Soc.*, 1941, **63**, 1737.

<sup>6</sup> C. S. Lu, E. W. Hughes, and P. A. Giguère, *ibid.*, p. 1507.

<sup>7</sup> W. H. Zachariasen, *J. Chem. Physics*, 1933, **1**, 634.

<sup>8</sup> S. B. Hendricks, *Amer. J. Sci.*, 1927, **14**, 269; J. West, *Z. Krist.*, 1930, **74**, 306.

<sup>9</sup> L. Helmholtz and R. Levine, *J. Amer. Chem. Soc.*, 1942, **64**, 354.

<sup>10</sup> L. Helmholtz, *ibid.*, 1937, **59**, 2036.

<sup>11</sup> W. H. Zachariasen and R. C. L. Mooney, *J. Chem. Physics*, 1934, **2**, 34.

<sup>12</sup> P. A. Giguère and V. Schomaker, *J. Amer. Chem. Soc.*, 1943, **65**, 2025.

<sup>13</sup> V. Schomaker and R. Spurr, *ibid.*, 1942, **64**, 1184.

<sup>14</sup> *E.g.*, L. Pauling, *op. cit.*, p. 304; T. G. Heafield and L. Hunter, *J.*, 1942, 420.

<sup>15</sup> S. H. Bauer, J. Y. Beach, and J. H. Simons, *J. Amer. Chem. Soc.*, 1939, **61**, 19.

<sup>16</sup> P. Günther, K. Holm, and H. Strunz, *Z. physikal. Chem.*, 1939, B, **43**, 229.

<sup>17</sup> L. Pauling and L. O. Brockway, *Proc. Nat. Acad. Sci.*, 1934, **20**, 336; J. Karle and L. O. Brockway, *J. Amer. Chem. Soc.*, 1944, **66**, 574.

\* See footnote, p. 141.

compounds or even isomers, in which such a structure is impossible or unlikely. Differences, if of sufficiently marked character, are then attributed to the hydrogen-bond structure of the former.

(a) *Infra-red absorption spectra*.\* This method is of very wide utility, for it can be applied to vapours, solutions, pure liquids, and even solids. It depends on the fact that the frequency and intensity of the infra-red absorption band characteristic of the group A-H undergo modification (to a lower frequency) when the hydrogen atom is involved in hydrogen-bond formation, as in A-H-B. Such shifts of the fundamental frequency may therefore be used as criteria of hydrogen bonding, although it has been emphasised<sup>18</sup> that they can sometimes be due to other causes. Though of recent development, this is rapidly becoming one of the most versatile diagnostic tests for hydrogen-bond structure, and already the presence of hydrogen bonds in a large number of compounds whose constitution had been deduced from other evidence has received confirmation by this method.<sup>19</sup> An important aspect is that, provided the portion of the spectrum under observation is not complicated by contributions due to other components of the molecule, this method, unlike the diffraction methods mentioned previously, is independent of molecular complexity and has, for example, provided the first experimental evidence, long anticipated on other grounds, of N-H-O bonds in proteins<sup>20</sup> and O-H-O bonds in cellulose.<sup>21</sup> Moreover, the method is capable of high sensitivity and can detect weak hydrogen bonds such as frequently operate in solute-solvent interactions;<sup>22</sup> indeed it is the only direct means at present available of demonstrating such transient compound-formation, and is even claimed to give indication of a preferred *cis*-configuration in *o*-chlorophenol<sup>23</sup> and in certain other *o*-substituted phenols (I),<sup>24</sup> in which, owing to the partial double-bond character of the O-nuclear link, geometrical isomerism of a rather transient type arises.

(b) *Volatility*. The effect of replacing the hydrogen atom responsible for intermolecular association in markedly reducing the boiling point of a compound has long been recognised; comparisons between the boiling points of alcohols and their methyl ethers, or between carboxylic acids and their methyl esters, provide good examples, and the phenomenon has

<sup>18</sup> A. M. Buswell, J. R. Downing, and W. H. Rodebush, *J. Amer. Chem. Soc.*, 1940, **62**, 2759.

<sup>19</sup> *E.g.*, see L. Pauling, *op. cit.*, p. 316 *et seq.*

<sup>20</sup> A. M. Buswell, K. F. Krebs, and W. H. Rodebush, *J. Physical Chem.*, 1940, **44**, 1126.

<sup>21</sup> J. W. Ellis and J. Bath, *J. Amer. Chem. Soc.*, 1940, **62**, 2859.

<sup>22</sup> W. Gordy, *J. Amer. Chem. Soc.*, 1938, **60**, 605; A. M. Buswell, W. H. Rodebush, and M. F. Roy, *ibid.*, p. 2528; W. Gordy and S. C. Stanford, *ibid.*, 1940, **62**, 497; W. Gordy, *J. Chem. Physics*, 1939, **7**, 93, 163, 167; W. Gordy and P. C. Martin, *ibid.*, p. 99; W. Gordy and S. C. Stanford, *ibid.*, 1940, **8**, 170.

<sup>23</sup> L. Pauling, *J. Amer. Chem. Soc.*, 1936, **58**, 94.

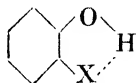
<sup>24</sup> O. R. Wulf, U. Liddel, and S. B. Hendricks, *ibid.*, p. 2287.

\* For a detailed review of work up to 1938, see G. B. B. M. Sutherland, *Ann. Reports*, 1938, **35**, 38.

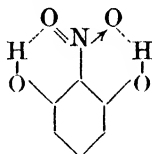


recently been used to demonstrate a hydrogen-bond structure in pyrazoles,<sup>25</sup> cyanamides,<sup>26</sup> and ethyleneimine.<sup>27</sup>

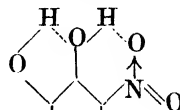
In the case of isomeric compounds, that isomer which can achieve an intramolecular hydrogen-bond structure is markedly more volatile than those which cannot.<sup>28</sup> Volatility in steam follows similar lines.<sup>29</sup> W. Baker<sup>30</sup> has drawn attention to the remarkable behaviour of 2-nitroresorcinol (II) and 3-nitrocatechol (III), which have boiling points *lower* than those of the unsubstituted phenols, and has attributed the effect to double chelation.



(I.)



(II.)



(III.)

(c) *Solubility and related properties.* Compounds possessing an intermolecular hydrogen-bond structure are usually more soluble in donor solvents (water, alcohol, pyridine, etc.) and less soluble in solvents of hydrocarbon type (light petroleum, benzene, carbon tetrachloride, etc.) than similar compounds (or isomers) in which such a structure is lacking. This effect is due to the fact that donor solvents (but not others) tend to simplify such solute molecules by replacing solute-solute bonds by solute-solvent bonds. On the other hand, an intramolecular (chelate) hydrogen-bond structure will usually survive solution even in a donor solvent, so that a compound of this type will be more soluble in hydrocarbon-like solvents, and less soluble in donor solvents, than its isomers in which a chelate structure is impossible. Much data of this kind is summarised by N. V. Sidgwick and R. K. Callow<sup>31</sup> and by N. V. Sidgwick.<sup>32</sup>

Solubility studies indicating solute-solvent interaction have been made by M. J. Copley, C. S. Marvel, and co-workers, pointing to the engagement of the hydrogen atom of di- and tri-halogenomethanes,<sup>33</sup> and of acetylenes<sup>34</sup> in bond formation with donor solvents. Similar conclusions have been drawn from heats of mixing.<sup>35</sup>

<sup>25</sup> H. T. Hayes and L. Hunter, *J.*, 1941, 1.

<sup>26</sup> L. Hunter and H. A. Rees, *J.*, 1945, 617.

<sup>27</sup> H. W. Thompson and G. P. Harris, *J.*, 1944, 301.

<sup>28</sup> N. V. Sidgwick and R. K. Callow, *J.*, 1924, 527; N. V. Sidgwick and T. W. J. Taylor, *J.*, 1922, 1853.

<sup>29</sup> N. V. Sidgwick and W. M. Aldous, *J.*, 1921, 1001.

<sup>30</sup> *J.*, 1934, 1684.

<sup>31</sup> See ref. 28.

<sup>32</sup> *J.*, 1925, 907.

<sup>33</sup> G. F. Zellhoefer, *Ind. Eng. Chem.*, 1937, **29**, 584; G. F. Zellhoefer, M. J. Copley and C. S. Marvel, *J. Amer. Chem. Soc.*, 1938, **60**, 1337; M. J. Copley, G. F. Zellhoefer, and C. S. Marvel, *ibid.*, p. 2666, 2714; 1940, **62**, 227; C. S. Marvel, F. C. Dietz, and M. J. Copley, *ibid.*, 1940, **62**, 2273.

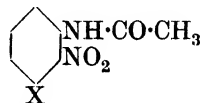
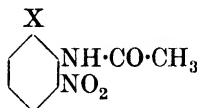
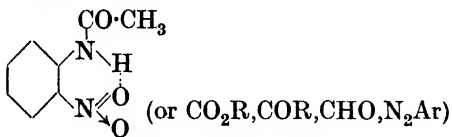
<sup>34</sup> M. J. Copley and C. E. Holley, *ibid.*, 1939, **61**, 1599; C. S. Marvel, J. Harkema, and M. J. Copley, *ibid.*, 1941, **63**, 1609.

<sup>35</sup> G. F. Zellhoefer and M. J. Copley, *ibid.*, 1938, **60**, 1343; M. J. Copley, C. S. Marvel and E. Ginsberg, *ibid.*, 1939, **61**, 3161; C. S. Marvel, M. J. Copley, and E. Ginsberg, *ibid.*, 1940, **62**, 3109, 3263; L. F. Audrieth and R. Steinman, *ibid.*, 1941, **63**, 2115.

The depression of melting point caused in the presence of liquid water ("wet melting-points") has been extensively applied by W. Baker<sup>36</sup> and later by H. O. Chaplin and L. Hunter<sup>37</sup> as a guide to chelate hydrogen-bond structure. The method is strictly applicable only to comparison of isomers, and is a rough determination of a triple point in the solubility (C.S.T.) diagram.<sup>38</sup> Compounds of chelate hydrogen-bond structure show a much smaller depression of melting point than their non-chelate (associated) isomers; *e.g.*, of the nitrophenols the (chelate) *o*-isomer shows a depression of melting point under water of 1°, but the (non-chelate) *m*- and *p*-isomers show depressions of 56° and 74° respectively.

(d) *Molecular weight.* The first systematic study of the connexion between molecular association and chemical structure was made in the last decade of the last century mainly by E. Beckmann and by K. von Auwers.<sup>39</sup> A useful review of this and other early work and an interpretation in terms of the hydrogen bond has been given by E. N. Lassettre.<sup>40</sup>

Molecular association, detected principally by the cryoscopic method in benzene and naphthalene solution, has recently been demonstrated in a large variety of organic types by L. Hunter and his collaborators, whose work<sup>41</sup> has revealed that the commonest single cause of molecular association in organic compounds is that due to intermolecular hydrogen bonding. Suppression of intermolecular hydrogen bonds by preferential intramolecular hydrogen bonding between suitably placed groups, first proposed by N. V. Sidgwick and R. K. Callow,<sup>28</sup> has now been detected in a large number of examples, as in the *o*-substituted acetanilides represented in (IV). Evidence is also adduced<sup>42</sup> that the chelate ring in (IV) is approximately co-planar with the benzene nucleus [a conclusion which would be expected as a result of conjugation of the groups in (IV) with the benzene ring], for the introduction of a substituent in the 6-position, as in (V), if large enough to be capable of interference with the acetyl group, will rotate the *N*-nuclear bond to such an extent that the imino-hydrogen atom can no longer achieve chelate ring-formation, and is therefore free to form inter-



molecular bonds; (V) is consequently associated. A similar result<sup>43</sup> follows from substitution in position 3, as in (VI), but here the interference

<sup>36</sup> *J.*, 1934, 1684; W. Baker and A. R. Smith, *J.*, 1936, 346; W. Baker, *J.*, 1937, 476; W. Baker and G. N. Carruthers, *J.*, 1937, 479.

<sup>37</sup> *J.*, 1938, 375, 1034; 1939, 484.

<sup>38</sup> N. V. Sidgwick, W. J. Spurrell, and T. E. Davies, *J.*, 1915, 1202.

<sup>39</sup> For collected references see K. v. Auwers, *Ber.*, 1937, 70, 966.

<sup>40</sup> *Chem. Reviews*, 1937, 20, 259.

<sup>41</sup> For collected references see *J.*, 1945, 806.

<sup>42</sup> H. O. Chaplin and L. Hunter, *J.*, 1938, 375.

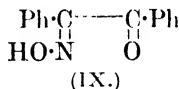
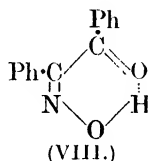
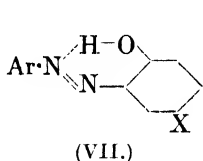
<sup>43</sup> *Idem, ibid.*, p. 1034.

has the effect of rotating the nitro-group so that its oxygen atoms are no longer co-planar with the benzene nucleus.

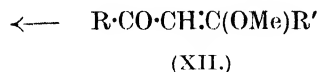
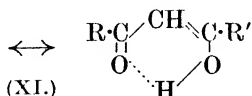
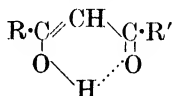
(e) *Miscellaneous*. (The symbol  $\longleftrightarrow$  is used in this and subsequent sections to denote molecular states between which resonance occurs.)

Under this heading is included a variety of properties, mainly chemical, in which compounds possessing a hydrogen-bond structure differ from those otherwise closely related to them. Whilst it cannot positively be stated that these differences are due to the hydrogen-bond structure, it is reasonable to quote them in support of it.

Groups involved in hydrogen-bond formation frequently suffer, as a result of their modified structure, a partial or complete suppression of their characteristic chemical properties. Examples of this effect are so numerous that only a few can be considered. *o*-Hydroxyazo-compounds (VII), unlike their *p*-isomers, are frequently insoluble in alkali and incapable of methylation by diazomethane;<sup>44</sup> they acylate with difficulty, and react only slowly with phenyl isocyanate. All these characters, as well as their mordant dyeing properties and their ability to form chelate metallic derivatives,<sup>45</sup> are no doubt consequent on the structure (VII) assigned to them. Of the two benzilmonoximes (VIII and IX), the one possessing the greater solubility in organic solvents and showing much reduced carbonyl activity is shown, by its derivation from triphenylisooxazole by oxidative fission,<sup>46</sup> to possess a configuration (VIII) capable of chelate ring formation.<sup>47</sup>



The fact that two enol forms of an unsymmetrical  $\beta$ -diketone have never been isolated has been used<sup>48</sup> as evidence supporting the hydrogen-bond structure (XI) of these compounds. In this connexion the demethyl-



ation of two isomeric enol ethers (X and XII; R = *o*-methoxyphenyl, R' = mesityl) to give a single enol<sup>49</sup> carries more conviction than formerly,

<sup>44</sup> C. Smith and A. D. Mitchell, *J.*, 1908, **93**, 843. The resistance of certain chelate *o*-hydroxyketones to methylation by diazomethane is discussed by A. Schönberg and A. Mustafa, *J.*, 1946, 746.

<sup>45</sup> (Miss) M. Elkins and L. Hunter, *J.*, 1935, 1598; H. D. K. Drew and J. K. Landquist, *J.*, 1938, 292.

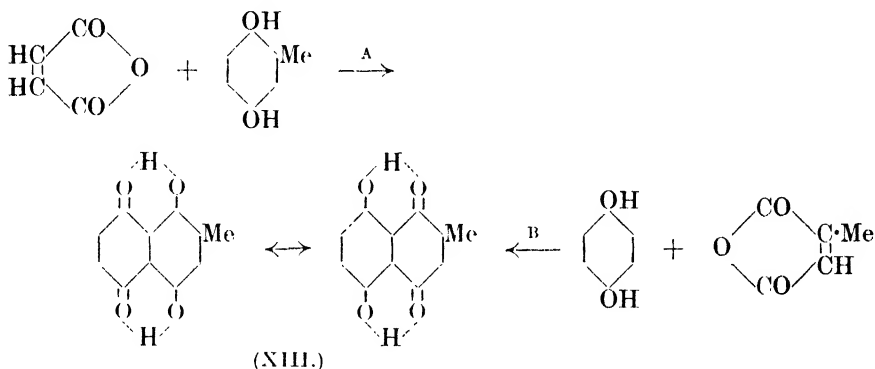
<sup>46</sup> J. Meisenheimer, *Ber.*, 1921, **54**, 3206.

<sup>47</sup> T. W. J. Taylor and E. K. Ewbank, *J.*, 1926, 2821.

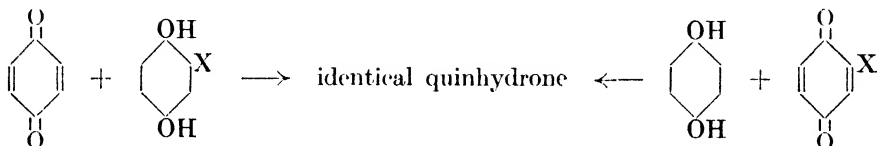
<sup>48</sup> N. V. Sidgwick, *Ann. Reports*, 1933, **30**, 113; 1934, **31**, 41.

<sup>49</sup> R. P. Barnes and C. C. Cochrane, *J. Amer. Chem. Soc.*, 1942, **64**, 2262.

since the product is a solid, m. p  $105^\circ$ , shown by titration to be 100% enol. There are numerous similar examples of the synthesis of a single individual by two alternative routes which, were it not for some identity-promoting influence such as hydrogen-bond formation, would be expected to lead to two different isomers. For example, methylnaphthazarin (XIII), only one isomer of which is known, can be synthesised<sup>50</sup> either by condensing maleic anhydride with methylquinol (Route A) or by condensing citraconic anhydride with quinol (Route B). Independent evidence supporting the

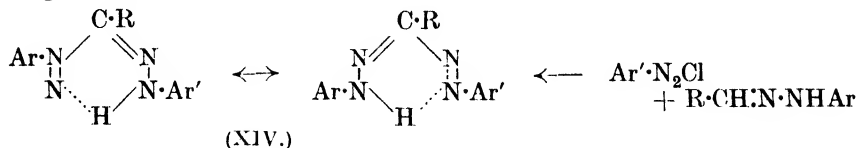


hydrogen-bond structure of naphthazarin has been provided from absorption spectra<sup>51</sup> and from crystal structure.<sup>52</sup> The above example is an intramolecular analogue of an unsymmetrical quinhydrone synthesised by the following two routes:



and a similar observation has been made in the case of the phenazhydrins.<sup>53</sup> A further example, which has recently been re-examined by L. Hunter and

$\text{Ar}\cdot\text{N}_2\text{Cl} + \text{R}\cdot\text{CH}\cdot\text{N}\cdot\text{NHA}\text{r}' \longrightarrow$



C. B. Roberts,<sup>54</sup> is provided by the formazyl compounds (XIV). It must be emphasised that, except in the case of quinhydrones, where there is

<sup>50</sup> A. K. Macbeth, J. R. Price, and F. L. Winzor, *J.*, 1935, 333.

<sup>51</sup> R. A. Morton and W. T. Earlam, *J.*, 1941, 159.

<sup>52</sup> J. Palačios and R. Salvia, *Anal. Fis. Quim.*, 1934, **32**, 49.

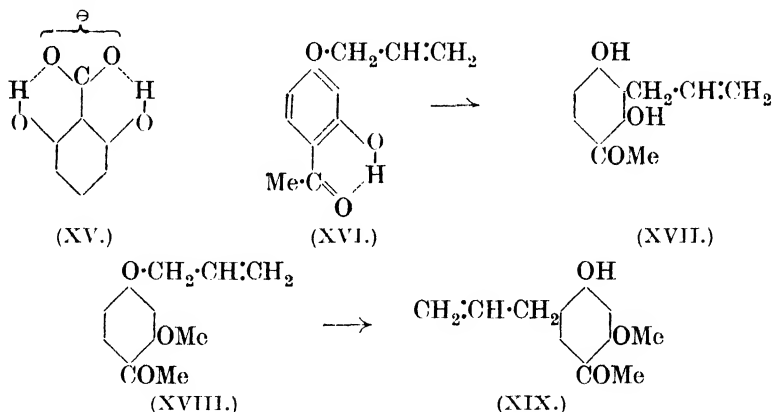
<sup>53</sup> G. R. Clemo and H. McIlwain, *J.*, 1934, 1991.

<sup>54</sup> *J.*, 1941, 820, 823.

independent evidence of hydrogen-bond structure,<sup>55</sup> the evidence of alternative synthesis quoted above cannot be taken as a rigid proof of hydrogen-bond structure because the possibility of prototropic conversion cannot be excluded.

The effect of chelation in stabilising a carboxylate anion (XV) has been postulated by W. Baker<sup>56</sup> to account for the greatly enhanced ionisation constant of 2:6-dihydroxybenzoic acid; such a structure would be expected to possess a much reduced proton attraction. In favour of this he points out the strong formal resemblance of this structure to 2-nitro-resorcinol (II). Similar structures involving only one carboxyl oxygen in hydrogen-bond formation have been proposed for the salicylate ion,<sup>57</sup> and to account for the abnormal basic strength of acridine-1-carboxylic acid.<sup>58</sup>

The partial fixation of one Kekulé structure in the benzene nucleus as a result of the engagement of two ortho-groups in hydrogen-bond formation, and its influence on group migration, has been the subject of study by W. Baker and (Miss) O. M. Lothian.<sup>59</sup> Thus, whereas 4-*O*-allylres-acetophenone (XVI) undergoes Claisen rearrangement to 3-allylres-acetophenone (XVII), its methyl ether (XVIII), in which no chelation is possible, gives the more normal 2-*O*-methyl-5-allylresacetophenone (XIX).



In conformity with modern views the authors now regard this effect as being due to a predominating contribution from the Kekulé form (XVI) to the structure of the resonance hybrid.<sup>59a</sup> Similar effects are discovered in other derivatives of resorcinol.<sup>60</sup>

Ability to form chelate metallic derivatives is usually an important

<sup>55</sup> O. R. Foz and J. Palaçios, *Anal. Fis. Quím.*, 1932, **30**, 421. Later work (J. Palaçios and O. R. Foz, *ibid.*, 1935, **33**, 627; 1936, **34**, 779) indicates that the hydrogen bond is not symmetrical, and that the quinone and quinol do not entirely lose their individuality in the crystal.

<sup>56</sup> *Nature*, 1936, **137**, 236.

<sup>57</sup> G. E. K. Branch and D. L. Yabroff, *J. Amer. Chem. Soc.*, 1934, **56**, 2568.

<sup>58</sup> A. Albert and R. Goldacre, *J.*, 1946, 710.

<sup>59</sup> *J.*, 1935, 628; 1936, 274.

<sup>59a</sup> Private communication.

<sup>60</sup> W. Baker, *J.*, 1934, 1684.

indication of *intra*-molecular hydrogen-bond structure. There is such a wealth of examples of this behaviour that it would be invidious to make any selection.<sup>61</sup> The property provides a popular basis for the qualitative and quantitative determination of many metals,<sup>62</sup> and is also the basis of Pfeiffer's stannic chloride test for chelate hydroxy-ketones and -quinones.<sup>63</sup> It is unsafe to assume, however, that inability to form chelate metallic derivatives indicates absence of hydrogen-bond structure in the parent compound. For example, the *o*-substituted derivatives of acetanilide (IV) mentioned on p. 145 do not yield metallic derivatives under ordinary conditions, presumably because of insufficient acidity in the imino-hydrogen atom concerned. Moreover, the type of co-ordination present in the metallic derivatives is not always structurally similar to that in the parent hydrogen compound; *e.g.*, diazoamino-compounds, which have been shown to possess an intermolecular hydrogen-bond structure,<sup>64</sup> yield metallic derivatives possessing an intramolecular structure.<sup>65</sup>

The profound changes in physical properties (elastic modulus, hardness, moisture sorption) brought about by *N*-methylation of polyamides of the nylon type support the evidence from other sources that these fibres consist of main chains cross-linked by N-H-O bonds.<sup>66</sup>

#### *The Types of Hydrogen Bond.*

Examples of the hydrogen bond are now very numerous and are still rapidly multiplying. It has long been known that increasing acidity in the hydrogen atom enhances its tendency to form hydrogen bonds, and a discovery which has led to a considerable expansion of the number of examples of the hydrogen bond is that tautomeric hydrogen, except when attached to carbon, is peculiarly liable to hydrogen-bond formation.<sup>41</sup> There is little doubt that this accounts for the surprising rarity of hydrogen bonds involving fluorine, in spite of the fact that this element forms the strongest known hydrogen bonds; for fluorine, unlike the multivalent elements oxygen, nitrogen, and sulphur, is unable to utilise hydrogen, whether attached to or juxtaposed to it, in protropic change.

F-H-F.—This bond is responsible for the considerable association of hydrogen fluoride,<sup>15, 16</sup> and for the occurrence and stability of acid fluorides. The F-F distance in the [F-H-F]<sup>-</sup> ions of potassium hydrogen fluoride (2.26 Å.)<sup>67</sup> and ammonium hydrogen fluoride (2.32 Å.)<sup>68</sup> are the shortest hydrogen bonds yet encountered.

<sup>61</sup> Many are listed in "Inorganic Chemistry: A Survey of Modern Developments", by (Sir) G. T. Morgan and F. H. Burstall, Heffer, Cambridge, 1936.

<sup>62</sup> "Organic Reagents for Metals", Hopkin and Williams, Ltd., London, 3rd Edn., 1938.

<sup>63</sup> P. Pfeiffer, P. Fischer, J. Kuntner, P. Monti, and Z. Pros, *Annalen*, 1913, **398**, 137.

<sup>64</sup> L. Hunter, *J.*, 1937, 320.

<sup>65</sup> A. Mangini and I. Dejudicibus, *Gazzetta*, 1933, **63**, 601; G. W. Watt and W. C. Fernelius, *Z. anorg. Chem.*, 1934, **221**, 187.

<sup>66</sup> W. O. Baker and C. S. Fuller, *J. Amer. Chem. Soc.*, 1943, **65**, 1120.

<sup>67</sup> L. Helmholz and M. T. Rogers, *ibid.*, 1939, **61**, 2590.

<sup>68</sup> M. T. Rogers and L. Helmholz, *ibid.*, 1940, **62**, 1533.

F-H-N.—The fact that crystalline ammonium fluoride follows the wurtzite pattern and thus differs in form from the other alkali and ammonium halides is a consequence of this bond; each nitrogen atom is surrounded tetrahedrally by four fluoride ions at a distance of 2.66 Å.<sup>69</sup> Crystalline ammonium hydrogen fluoride exemplifies hydrogen bonds of two distinct types and lengths; not only are the fluorine atoms arranged tetrahedrally around the nitrogen atom (F-H-N = 2.80 Å.) but they are themselves paired by a connecting bond (F-H-F = 2.32 Å.).<sup>70</sup> The crystal structure of hydrazinium difluoride is similarly dominated by the F-H-N bond (F-H-N = 2.62 Å.).<sup>71</sup>

F-H-O.—There is no evidence for this bond beyond that provided by the infra-red absorption of *o*-fluorophenol, which has been interpreted<sup>24</sup> as indicating a chelate structure (I; X = F). X-Ray examination of the hydrated fluorides and complex fluorides of the Group I metals, long overdue, may eventually provide further examples of this bond.

O-H-O.—This type provides the most abundant examples of the hydrogen bond, and is responsible for the molecular association of water, alcohols, phenols, and carboxylic acids. It is probable that in this connexion the carboxylic acids are a special case of a much wider class exhibiting hydrogen bond structure, *viz.*, the oxy-acids, although this has been confirmed only for boric,<sup>72</sup> iodic,<sup>73</sup> and telluric<sup>74</sup> acids. Even though a proportion of the acidic hydrogen in an oxy-acid is replaced (*e.g.*, by metal), the remaining oxy-anion shows clear indication of hydrogen-bond structure, as in sodium hydrogen carbonate,<sup>7</sup> potassium dihydrogen phosphate<sup>8</sup> and arsenate,<sup>9</sup> and ammonium trihydrogen paraperiodate.<sup>10</sup>

The bond plays an important part in the structure of hydrated salts, especially where the hydrated water is associated with an oxy-anion.<sup>75</sup> Considerable clarification of the structure of metallic hydroxides, including certain minerals, has resulted from the recognition of their hydrogen-bond structure, and many such examples have been reviewed by J. D. Bernal and (Miss) H. D. Megaw,<sup>76</sup> who designate the rather weak O-H-O bonds manifested in these compounds as "hydroxyl bonds" (see under "Nomenclature", p. 155).

The profound influence of the hydrogen bond in deciding in many cases not only the crystal habit but many other physical properties of crystals, may be illustrated by numerous striking examples. By maintaining an open structure within the crystal of the ordinary ( $\alpha$ -) form of resorcinol,

<sup>69</sup> W. H. Zachariasen, *Z. physikal. Chem.*, 1927, **127**, 218.

<sup>70</sup> M. T. Rogers and L. Helmholz, *J. Amer. Chem. Soc.*, 1940, **62**, 1533.

<sup>71</sup> M. L. Kronberg and D. Harker, *J. Chem. Physics*, 1942, **10**, 309.

<sup>72</sup> W. H. Zachariasen, *Z. Krist.*, 1934, **88**, 150.

<sup>73</sup> M. T. Rogers and L. Helmholz, *J. Amer. Chem. Soc.*, 1941, **63**, 278.

<sup>74</sup> B. Gossner and O. Kraus, *Z. Krist.*, 1934, **88**, 298; L. Pauling, *ibid.*, 1935, **91**, 367.

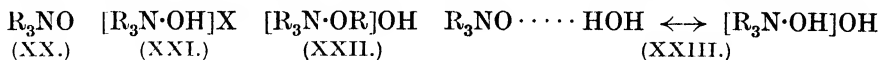
<sup>75</sup> A detailed review of such hydrates is given by A. F. Wells, "Structural Inorganic Chemistry", Oxford Univ. Press, 1945, pp. 364 *et seq.*

<sup>76</sup> *Proc. Roy. Soc.*, 1935, *A*, **151**, 384; J. D. Bernal and A. F. Wells, *Ann. Reports*, 1935, **32**, 212.

intermolecular hydrogen bonds prevent the most economical packing of the molecules and result in a crystal of density 1.28. At temperatures above about 74° a slow transition occurs to a polymorphous ( $\beta$ -) form ( $d$  1.33) in which the molecules achieve a somewhat closer union by a rearrangement of the hydrogen bonds.<sup>77</sup> The formation of layers in certain crystals by means of O-H $\cdots$ O bonds is a much favoured arrangement, especially where the molecules are planar or nearly so. An example of this is provided by crystalline boric acid, the molecules of which are united in infinite layers by hydrogen bonds, neighbouring layers adhering only by residual forces,<sup>72</sup> thus accounting for the softness and perfect basal cleavage of boric acid crystals. The easy layer cleavage of the  $\alpha$ -form of anhydrous oxalic acid is due to a similar cause, although in the  $\beta$ -form, where the molecules are linked in chains, cleavage into strips or laths parallel to the axes of the chains occurs. The dominating influence of the hydrogen bond in determining the particularly open structure of ice, and the anomalous behaviour of the water-ice system in the neighbourhood of 0°, has been discussed by J. D. Bernal and R. H. Fowler.<sup>78</sup> This unique behaviour has incalculable consequences in biological as well as in physical phenomena.

Among organic compounds, the common property of molecular association in all compounds containing hydroxyl groups is due to O-H $\cdots$ O bonds. Where the hydroxyl group is favourably placed to receive electrons from a donor oxygen *within* the molecule, intramolecular O-H $\cdots$ O bonds may be set up as part of a chelate ring system. This occurs in enolised  $\beta$ -keto-esters and  $\beta$ -diketones (XI), in phenols substituted in the *o*-position by groups such as NO<sub>2</sub>, CHO, COR, CO<sub>2</sub>H, CO<sub>2</sub>R, and OH, and in many other types.

The puzzling behaviour of the amine oxides (XX) has received satisfactory explanation on the basis of the O-H $\cdots$ O bond.<sup>79</sup> In aqueous solution these compounds form weakly ionised hydroxides, and their salts (XXI) are correspondingly strongly hydrolysed in aqueous solution. On the contrary the quaternary hydroxides derived from the related hydroxylamines (XXII) are strong bases yielding neutral, highly ionised salts.<sup>80</sup>



This striking difference between the two types of hydroxide is due to the assumption by the hydrated amine oxides of an O-H $\cdots$ O structure (XXIII) which largely reduces their effective basic character; (XXII) cannot assume a corresponding structure, and is consequently very highly ionised.

N-H $\cdots$ O.—Though not so named at the time, the earliest examples of the hydrogen bond were of this type. In 1906, anomalies in the behaviour

<sup>77</sup> J. M. Robertson and A. R. Ubbelohde, *Proc. Roy. Soc.*, 1938, A, **167**, 122.

<sup>78</sup> *J. Chem. Physics*, 1933, **1**, 515.

<sup>79</sup> G. N. Lewis, "Valence and the Structure of Atoms and Molecules", Chemical Catalog Co., New York, 1923, p. 111.

<sup>80</sup> T. D. Stewart and S. Maeser, *J. Amer. Chem. Soc.*, 1924, **46**, 2583.



of certain *o*-hydroxyazo-compounds led G. Oddo<sup>81</sup> to propose for them a structure closely related to (VII), and a few years later T. S. Moore and T. F. Winmill<sup>82</sup> invoked its aid to explain the incomplete ionisation of the hydroxides present in aqueous solutions of aliphatic amines. Much later, its presence was inferred from molecular-weight studies of oximes,<sup>83</sup> amides and sulphonamides,<sup>84</sup>  $\alpha$ -piperidone,<sup>85</sup> and numerous examples of its intramolecular operation in *o*-substituted anilides (IV) and certain *o*-substituted phenols (I—III) have already been referred to. X-Ray analyses have indicated the N—H—O bond in acetamide,<sup>86</sup> urea,<sup>87</sup> amino-acids,<sup>88</sup> diketopiperazine,<sup>89</sup> and polypeptides,<sup>90</sup> and it is on the authority of such measurements that hydrogen bonds of the N—H—O type have been postulated as a fundamental link in the structure of proteins. This has now been confirmed for sixteen proteins by infra-red spectroscopy.<sup>20</sup>

N—H—N.—Because this bond was at first confined to crystalline ammonia (N—H—N = 3.38 Å.),<sup>91</sup> and to ammonium azide (N—H—N = 2.96 Å.)<sup>92</sup> it was considered to be both rare and very weak. This view must be abandoned since the bond has now been demonstrated, by X-ray analysis, in phthalocyanine,<sup>4</sup> melamine,<sup>5</sup> and dicyanodiamide,<sup>93</sup> and by infra-red spectroscopy in ethyleneimine,<sup>27</sup> and has been shown to be responsible for the molecular association of diazoamino-compounds,<sup>94</sup> triazoles,<sup>95</sup> amidines, iminazoles and guanidines,<sup>96</sup> pyrazoles and indazoles,<sup>97</sup> and cyanamides.<sup>98</sup> It is also present in certain chelate ring systems, including formazyl compounds,<sup>54</sup> and *o*-aminoazo-compounds<sup>99</sup> and their acyl<sup>100</sup> and thioacyl<sup>101</sup> derivatives.

N—H—S.—Although the normal physical behaviour of hydrogen sulphide, thiols, and thiophenols, as compared with their oxygen analogues, is taken as evidence of the non-existence of the S—H—S bond, recent molecular-weight studies of the thioamides<sup>102</sup> have shown that these compounds closely resemble the amides in exhibiting molecular association, attribut-

<sup>81</sup> *Gazzetta*, 1906, **36**, ii, 1.

<sup>82</sup> *J.*, 1912, 1635.

<sup>83</sup> N. V. Sidgwick, *Ann. Reports*, 1934, **31**, 41.

<sup>84</sup> H. O. Chaplin and L. Hunter, *J.*, 1937, 1114.

<sup>85</sup> G. I. Jenkins and T. W. J. Taylor, *J.*, 1937, 495.

<sup>86</sup> F. Senti and D. Harker, *J. Amer. Chem. Soc.*, 1940, **62**, 2008.

<sup>87</sup> R. W. G. Wyckoff and R. B. Corey, *Z. Krist.*, 1934, **89**, 462.

<sup>88</sup> G. Albrecht and R. B. Corey, *J. Amer. Chem. Soc.*, 1939, **61**, 1087; H. A. Levy and R. B. Corey, *ibid.*, 1941, **63**, 2095.

<sup>89</sup> R. B. Corey, *ibid.*, 1938, **60**, 1598.

<sup>90</sup> E. W. Hughes and W. J. Moore, *ibid.*, 1942, **64**, 2236.

<sup>91</sup> H. Mark and E. Pohland, *Z. Krist.*, 1925, **61**, 532.

<sup>92</sup> L. K. Frevel, *J. Amer. Chem. Soc.*, 1936, **58**, 779.

<sup>93</sup> E. W. Hughes, *ibid.*, 1940, **62**, 1258. <sup>94</sup> L. Hunter, *J.*, 1937, 320.

<sup>95</sup> T. G. Heafield and L. Hunter, *J.*, 1942, 420.

<sup>96</sup> L. Hunter and J. A. Marriott, *J.*, 1941, 777.

<sup>97</sup> H. T. Hayes and L. Hunter, *J.*, 1941, 1.

<sup>98</sup> L. Hunter and H. A. Rees, *J.*, 1945, 617.

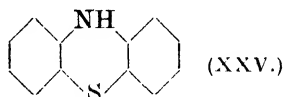
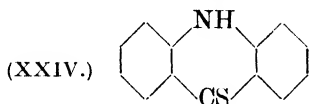
<sup>99</sup> A. Cremonini, *Gazzetta*, 1928, **58**, 372.

<sup>100</sup> H. O. Chaplin and L. Hunter, *J.*, 1938, 375.

<sup>101</sup> G. Hopkins and L. Hunter, *J.*, 1942, 638.

<sup>102</sup> *Idem*, *loc. cit.*; see also K. v. Auwers, *Z. physikal. Chem.*, 1899, **30**, 529.

able to a N-H-S structure. An important feature of the bond is that it is confined to compounds in which the sulphur atom can accept or part with hydrogen by tautomeric transfer; for example, though present in thio-



acridone (XXIV) it is absent in thiodiphenylamine (XXV). For this reason it will probably prove to be present in thioureas, thiocarbamic esters, etc., but not in proteins.

Interaction between thiophenol and pyridine and other bases, ascribed to weak N-H-S bonds, has been claimed on spectroscopic evidence.<sup>103</sup>

O-H-S.—Evidence for this bond is rather scanty and rests on molecular association in the thiocarboxylic acids<sup>104</sup> and on the existence of certain compounds of benzoquinone with thiols and with thiophenols<sup>105</sup> which bear a close resemblance to the quinhydrones. Anomalous heats of mixing of thiophenol with certain donor solvents have been attributed<sup>106</sup> to weak O-H-S bonds, and interaction between thiophenol and dioxan, detected by Raman spectroscopy,<sup>107</sup> seems to be due to the same cause.

*Hydrogen Bonds involving Carbon* (C-H-O, C-H-N).—So weak is the tendency of the CH group to form a hydrogen bond that it is manifested only under the influence of activating atoms or groups tending to promote the ionisation of the hydrogen atom, as in chloroform, hydrogen cyanide, and phenylacetylene. Even then, the bond is seldom strong enough to lead to anything but transient compound-formation, and has never, except perhaps in hydrogen cyanide, been detected by molecular weight methods. Nevertheless, the application of more sensitive methods gives clear indication of intermolecular bonding of a weak kind; for example, the high dielectric polarisation of mixtures of halogenoforms and certain donor liquids,<sup>108</sup> the anomalous solubilities of halogenoforms<sup>33</sup> and acetylenes<sup>34</sup> in donor solvents, infra-red absorption displacements of chloroform and phenylacetylene when dissolved in donor solvents,<sup>22</sup> anomalous heats of mixing of halogenoforms with donor liquids,<sup>35</sup> and the abnormal physical properties of hydrogen cyanide,<sup>109</sup> have all been attributed to weak C-H-O and C-H-N bonds.

The participation of the CH group in chelate hydrogen-bond formation has frequently been postulated to account for anomalies in certain *o*-disubstituted derivatives of benzene and in other compounds where such a

<sup>103</sup> W. Gordy and S. C. Stanford, *J. Amer. Chem. Soc.*, 1940, **62**, 497.

<sup>104</sup> K. v. Auwers, *loc. cit.*; T. G. Heafield, G. Hopkins, and L. Hunter, *Nature*, 1942, **149**, 218.

<sup>105</sup> Beilstein's Handbuch, 4th Edn., Vol. VII, pp. 615, 616, 646.

<sup>106</sup> M. J. Copley, C. S. Marvel, and E. Ginsberg, *J. Amer. Chem. Soc.*, 1939, **61**, 3161.

<sup>107</sup> R. H. Saunders, M. J. Murray, and F. F. Cleveland, *ibid.*, 1942, **64**, 1230.

<sup>108</sup> D. P. Earp and S. Glasstone, *J.*, 1935, 1709.

<sup>109</sup> L. Pauling, *op. cit.*, p. 294.

structure is sterically possible.<sup>110</sup> Thus, the superior volatility of *o*-nitro-toluene,<sup>28</sup> the high dissociation constant of *o*-toluic acid,<sup>111</sup> the low dissociation constant of *o*-nitrophenylacetic acid,<sup>112</sup> and the slow rate of hydrolysis of ethyl *o*-toluate,<sup>113</sup> when compared with their *m*- and *p*-isomers, have all been attributed to the formation of six-membered chelate rings involving the methyl or methylene groups in weak C-H-O bonds.

*Hydrogen Bonds involving Other Elements.*—Although strong hydrogen bonds are confined to the electronegative elements of the first short period, it is not surprising that their homologues, especially in the second short period, should also exhibit a similar though much weaker tendency. Only in sulphur has this tendency been shown to be marked,<sup>102</sup> although preliminary work (unpublished) on compounds of phosphorus points to the possibility that this element too may form weak hydrogen bonds.

In regard to the homologues of fluorine, reference has already been made to the spectroscopic evidence<sup>23</sup> for Cl-H-O bonds in *o*-chlorophenol, and it is noteworthy that the boiling point (176°) of this compound is about 40° below those of its *m*- and *p*-isomers. Similar evidence has been adduced<sup>114</sup> for weak Cl-H-O bonds in ethylene chlorohydrin, and for weak Br-H-O bonds in ethylene bromohydrin, *o*-bromophenol, and tetra-bromo-guaiacol and -catechol.<sup>24</sup>

The hydrogen-bond structure proposed<sup>115</sup> for the boron hydrides and certain of their mixed hydrides, though it may be deemed to fall within the definition given at the beginning of this article, differs markedly from the types previously considered, particularly as the boron (and certain metallic) atoms are linked simultaneously through two hydrogen atoms. The authors propose to emphasise the difference by using the term "resonance link" in connexion with such structures.

*Hydrogen-bond Association and Tautomerism.*—The work of L. Hunter and his collaborators<sup>41</sup> has revealed a close parallel between the tautomeric behaviour of a large number of organic types, such as amides, diazoamino-compounds, pyrazoles, glyoxalines, etc., and their molecular association, and it is suggested that both are due to the intermolecular sharing of the hydrogen atoms responsible for the tautomeric character of these types. A new classification is therefore proposed for systems whose tautomerism depends upon "mobile hydrogen". Those systems in which the alternative sites of attachment of the mobile hydrogen are oxygen, nitrogen, or sulphur (but not carbon) are now regarded as exhibiting "mesohydric tautomerism"; compounds whose tautomeric character depends on the mobility of hydrogen attached to carbon, by virtue of its inability to form stable hydrogen bonds

<sup>110</sup> See J. F. J. Dippy, *Chem. Reviews*, 1939, **25**, 151.

<sup>111</sup> J. F. J. Dippy, D. P. Evans, J. J. Gordon, R. H. Lewis, and H. B. Watson, *J.*, 1937, 1421.

<sup>112</sup> J. F. J. Dippy and R. H. Lewis, *ibid.*, p. 1426.

<sup>113</sup> D. P. Evans, J. J. Gordon, and H. B. Watson, *ibid.*, p. 1430.

<sup>114</sup> L. R. Zumwalt, and R. M. Badger, *J. Chem. Physics*, 1939, **7**, 87.

<sup>115</sup> H. C. Longuet-Higgins and R. P. Bell, *J.*, 1943, 250.

when thus combined, are excluded from this type of tautomerism. Dual character, which in true prototropy is due to a mixture of tautomers (or at least to a mesomeric ion common to both tautomers), is believed in the case of mesohydric tautomerism to be due to a homogeneous substance. Whether this is so or not, the concept is useful insofar as it directs attention to the futility of attempts to separate the tautomeric forms of the types included under mesohydric tautomerism.

*Nomenclature.*—Although the term *hydrogen bond* is well established in this country, American practice favours the term *hydrogen bridge*, whilst W. G. Palmer,<sup>116</sup> pointing out that the effective agent in all cases is the proton, advocates the term *proton bond*. J. D. Bernal's distinction<sup>117</sup> between a "true" hydrogen bond (in which only one of the two linked atoms possesses attached hydrogen) and the *hydroxyl bond* (in which both atoms have attached hydrogen), though useful in many respects, should not be allowed to obscure the fact that the difference is probably one of degree and not of kind, and that there is a continuous gradation with no sharp distinction, either in bond length or in properties, between the extremely weak and the strongest hydrogen bonds. A distinction of another kind has been proposed<sup>118</sup> for molecular association due to hydrogen bonds between like groups (homogeneous) and between unlike groups (heterogeneous), and it is shown that the enhancement of the molecular association of phenols and other associated compounds by the substitution of certain donor groups must be due in part to the latter type.

L. H.

### 3. STEREOCHEMISTRY.

A total asymmetric synthesis of ethyl *d*-tartrate has been reported by T. L. Davis and J. Ackerman.<sup>1</sup> A mixture of ethyl fumarate and anhydrous hydrogen peroxide in ethereal solution was irradiated with right-handed circularly polarised light of wave-band 2535—7—9 Å. Rotations of  $+0.073^\circ$  and  $+0.030^\circ$  (both  $\pm 0.003^\circ$ ) were observed. The rotation increased with time of irradiation up to 120 minutes but thereafter decayed rather rapidly to zero at 190 hours.

It was stated<sup>2</sup> that a specimen of santonin prepared synthetically was subsequently found to be optically active, although it was obtained from inactive materials. Later there was reported<sup>3</sup> the appearance of optical activity during the methylation of 2-formylcyclohexanone by means of methyl iodide and sodium, the figure  $[\alpha]_D -26.22^\circ$  being given for the rotation of the 2-formyl-2-methylcyclohexanone formed. C. S. Gibson<sup>4</sup>

<sup>116</sup> "Valency", Cambridge, 1945, p. 226.

<sup>117</sup> J. D. Bernal and (Miss) H. D. Megaw, *Proc. Roy. Soc.*, 1935, A, **151**, 384; J. D. Bernal, *Trans. Faraday Soc.*, 1940, **36**, 922.

<sup>118</sup> L. Hunter and J. A. Marriott, *J.*, 1940, 166.

<sup>1</sup> *J. Amer. Chem. Soc.*, 1945, **67**, 486.

<sup>2</sup> K. D. Paranjape, N. L. Phalnikar, B. V. Bhide, and K. S. Nargund, *Current Science*, 1943, **12**, 153.

<sup>3</sup> *Nature*, 1944, **153**, 141.

<sup>4</sup> *Ibid.*, p. 225.

pointed out that this claim should not be allowed to pass without careful examination, and the actual methylation process was examined experimentally in two other laboratories, an inactive product being obtained, as would be expected.<sup>5, 6</sup> The Oxford workers<sup>6</sup> referred to the known element of chance in such syntheses: it is clear that this factor could not permit of activities of the magnitude claimed by the Indian workers, and if they did indeed isolate an active product they must inadvertently have done one or other of the following things: (1) carried out the methylation in presence of singularly efficient circularly polarised light, (2) removed some of their product by selective extraction with an optically active solvent or by selective adsorption on an "asymmetric" adsorbent, (3) introduced an active second substance. It is to be hoped that the origin of the activity will be disclosed in due course.

Since diastereoisomerides differ in all physical properties, they should be separable by distillation, but it is only in comparatively recent years that the efficiency of stills has become high enough to permit of the realisation of this fact. M. E. Bailey and H. B. Hass<sup>7</sup> distilled *d*-2-methylbutyl methylethylacetate in a 60-plate Lecky column.<sup>8</sup> The first 40% and the last 30% of the distillate gave on saponification *l*- and *d*-methylethylacetic acid with  $[\alpha]_D^{25} - 0.25^\circ$  and  $+ 0.29^\circ$ , respectively. Similar results were obtained with other mixtures of diastereoisomerides. Thus with the *dl*-*sec*-butyl ester of *d*- $\alpha$ -propionoxypropionic acid and distilling at 35 mm. resolution was effected to such a high degree that one saponified fraction (the last 15% of the total distillate) gave *d*-*sec*-butyl alcohol of 86% optical purity.

Two resolutions are worth noting: that of racemic acid<sup>9</sup> using *d*-amphetamine, in which racemic acid and the *d*-base in aqueous solution gave an 85% yield of *d*-base hydrogen *l*-tartrate as the less soluble salt; and that of *dl*-biotin,<sup>10</sup> where use was made, rather unusually, of arginine; *l*(+)-arginine and *dl*-biotin in aqueous *isopropyl* alcohol gave arginine *d*-biotin in 92% yield, and this after crystallisation and treatment with dilute acid gave pure *d*-biotin.

It now appears to be fairly certain that the sulphur-oxygen link in sulfoxides is a double and not a co-ordinate one.<sup>11</sup> Similarly the phosphorus-oxygen bond in a phosphine oxide and the phosphorus-sulphur bond in a phosphine sulphide is a double bond. These bonds are all much too short to be of the type present in the amine oxides, these being proper dative bonds of length about equal, as would be required, to that of the equivalent covalent bond. The molecule of a sulphoxide is now to be represented as in *A*, which Sutton presumably meant when he used the term

<sup>5</sup> J. M. O'Gorman, *J. Amer. Chem. Soc.*, 1944, **66**, 1041.

<sup>6</sup> J. W. Cornforth, R. H. Cornforth, and M. J. S. Dewar, *Nature*, 1944, **153**, 317.

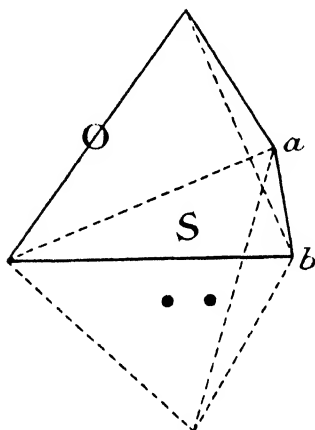
<sup>7</sup> *J. Amer. Chem. Soc.*, 1941, **63**, 1969.    <sup>8</sup> *Ind. Eng. Chem. Anal.*, 1940, **12**, 544.

<sup>9</sup> E. Walton, *J. Soc. Chem. Ind.*, 1945, **64**, 219.

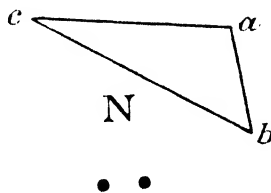
<sup>10</sup> D. E. Wolf, R. Mozingo, S. A. Harris, R. C. Anderson, and K. Folkers, *J. Amer. Chem. Soc.*, 1945, **67**, 2100.

<sup>11</sup> G. M. Phillips, J. S. Hunter, and L. E. Sutton, *J.*, 1945, 146.

"trigonal bipyramid". In fact, the configuration of a sulfoxide is little different under the new constitution from what it was with the co-ordinate bond structure. In the new configuration, the sulphur atom is situated with respect to groups *a* and *b* and the oxygen atom much as the nitrogen atom is situated with respect to the three groups *a*, *b* and *c* (see *B*) in a tertiary amine. The difference in optical stability between the sulfoxides and the amines may be due (1) to the larger size of the sulphur atom, (2) to the presence of the strong S:O bond, and possibly (3) to stabilising resonance between the electrons of the double bond and the pair of unshared electrons which "occupy" the lower half of the trigonal bipyramid.

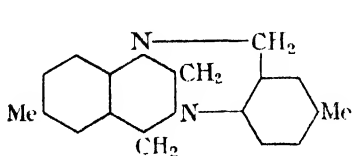


(A)

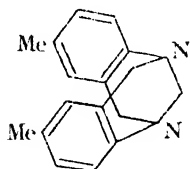


(B)

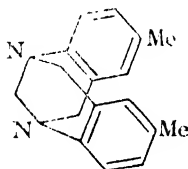
A condensation product of *p*-toluidine and formaldehyde known as Troeger's base was shown by M. A. Spielman<sup>12</sup> to be (I). V. Prelog and



(I.)



(II.)



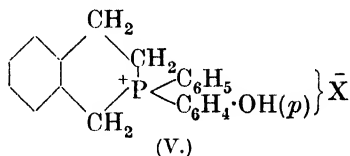
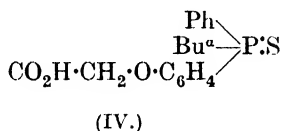
(III.)

P. Wieland<sup>13</sup> noticed that if the nitrogen atoms could assume a stable "tetrahedral" configuration the base should exist in mirror-image forms (II and III). Owing to the weakness of the base, resolution could not be attempted with tartaric acid, and, no doubt owing to the instability of the base to acids, only partial and erratic resolution was effected using camphor- and bromocamphor-sulphonic acids. Complete resolution was achieved by chromatographic adsorption using *d*-lactose hydrate activated by extraction

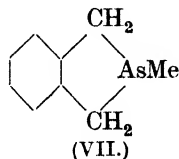
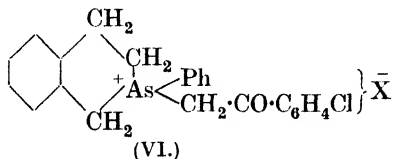
<sup>12</sup> *J. Amer. Chem. Soc.*, 1935, **57**, 533.<sup>13</sup> *Helv. Chim. Acta*, 1944, **27**, 1127.

with chloroform<sup>14</sup> followed by drying and, most essentially, grinding in a steel ball-mill. Using light petroleum as the solvent, the *d*-base was the more strongly adsorbed. The enantiomorphs were obtained with  $[\alpha]_D^{16.5} + 287^\circ \pm 7^\circ$  and  $-278^\circ \pm 7^\circ$  (in hexane). Although the active forms were rapidly racemised in alcoholic hydrochloric acid, they were little affected by sublimation in a high vacuum. This result is of considerable interest since it provides the first example of a practicable resolution based on chromatography. It hardly bears on the vexed question of the stereochemistry of tervalent nitrogen, since the molecule of Troeger's base has a very rigid structure and the resolution merely provides another rather unusual case of molecular dissymmetry. The rigidity, and to some extent the instability in presence of acid, recall the molecule of hexamine, and, in the first respect that of the quinuclidine portion of the cinchona alkaloids.

W. C. Davies and F. G. Mann<sup>15</sup> describe the sharp resolution of phenyl-*p*-(carboxymethoxy)phenyl-*n*-butylphosphine sulphide (IV) using *d*- and *l*-1-phenylethylamine. The active forms had  $[M]_D \pm 9.6^\circ$  in benzene solution, and showed complex anomalous rotatory dispersion with a maximum rotation at about  $\lambda$  5800. F. G. Holliman and F. G. Mann have achieved marked success by obtaining the cyclic phosphorus compound (V) in an active form.<sup>16</sup>



Since 1939, when the last report was made<sup>17</sup> on the stereochemistry of arsenic, a number of very interesting and important contributions have been made by F. G. Mann and his collaborators. Chemical evidence for the validity of the dissociation-equilibrium theory of the optical instability of arsonium salts<sup>18</sup> has been obtained by F. G. Holliman and F. G. Mann,<sup>19</sup> who have resolved into optically active forms 2-phenyl-2-*p*-chlorophenacyl-1:2:3:4-tetrahydroisoarsinolinium salts (VI). This particular arsonium salt was chosen because there was evidence that a *p*-chloro-atom stabilised the attachment of a phenacyl radical to the arsenic atom. Resolution



was effected through the bromocamphorsulphonates, from which the *d*- and *l*-picrates were obtained with  $[M]_D + 457^\circ$  and  $-450^\circ$ , respectively,

<sup>14</sup> G. M. Henderson and H. G. Rule, *J.*, 1939, 1568.

<sup>15</sup> *J.*, 1944, 276. <sup>16</sup> *Nature*, 1947, 159, 438.

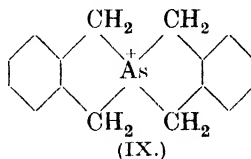
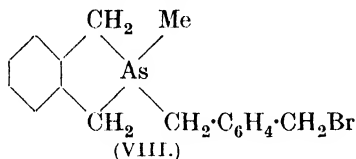
<sup>17</sup> *Ann. Reports*, 1939, 36, 236.

<sup>18</sup> G. J. Burrows and E. F. Turner, *J.*, 1921, 119, 426.

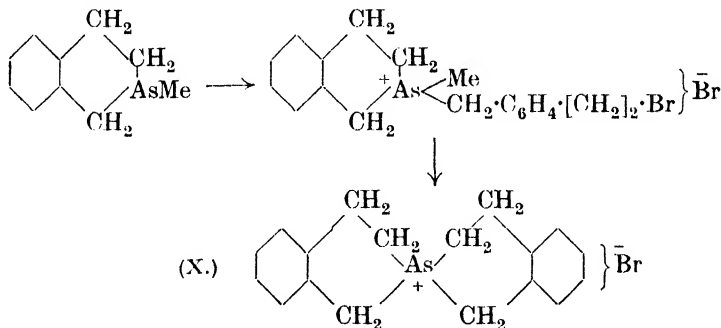
<sup>19</sup> *J.*, 1943, 550.

in chloroform. The active *l*-iodide, with  $[M]_D - 354^\circ$ , did not racemise at all in chloroform solution during five days at the ordinary temperature. This high optical stability taken in conjunction with the chemical stability of the *iso*arsinolinium salts provides strong evidence in favour of the dissociation theory of the optical instability of quaternary arsonium salts.

D. R. Lyon and F. G. Mann<sup>20</sup> have shown that 2-methyl*iso*arsindoline (VII) readily combines with one equivalent of *o*-xylylene dibromide to give 2-*o*-(bromomethyl)benzyl-2-methyl*iso*arsindolinium bromide (VIII) which, when heated, loses methyl bromide with the formation of the highly crystalline *As*-*spiro*bis*iso*arsindolinium bromide (IX). Using this interesting



method for the synthesis of spirocyclic arsonium salts, F. G. Holliman and F. G. Mann<sup>21</sup> have prepared *As*-*spiro*-bis-1 : 2 : 3 : 4-tetrahydro*iso*arsinolinium bromide (X) from 2-methyl-1 : 2 : 3 : 4-tetrahydro*iso*arsindoline and a valuable substance these authors have used in a number of ways, namely, *o*-2-bromoethylbenzyl bromide :



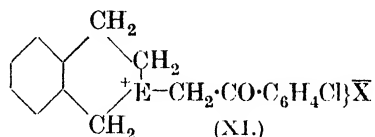
The cation of (X) possesses molecular dissymmetry. The bromocamphor-sulphonate was resolved and the active iodides isolated with  $[M]_D + 342^\circ$  and  $-344^\circ$  in chloroform solution, in which these salts had high optical stability. The authors point out that the resolution of this spirocyclic arsonium salt supports, but does not prove, the tetrahedral configuration of the 4-covalent arsenic atom. If the latter possessed a pyramidal configuration the spirocyclic salt (X) could give rise to geometrical isomerides, and the *trans*-form, having no element of symmetry, could show optical activity. Actually there was no evidence that two such forms existed.

Again making use of *o*-2-bromoethylbenzyl bromide, the same authors<sup>22</sup> have added to the known thio*iso*chroman the corresponding selenium and tellurium compounds and as a result have been able to prepare and resolve

<sup>20</sup> *J.*, 1945, 30.<sup>21</sup> *J.*, 1945, 45.<sup>22</sup> *J.*, 1945, 37.



a eutropic series of compounds (XI) in which E is S, Se, or Te. The actual salts resolved in each case were the bromocamphorsulphonates, which were converted into active picrates. 2-*p*-Chlorophenacylthioisochromanium picrate (E = S) was obtained with  $[M]_D - 242^\circ$  and  $+ 250^\circ$  in acetone. The corresponding seleno-picrate (E = Se) had  $[M]_D - 533^\circ$  and  $+ 504^\circ$  in acetone. Both sets of picrates were optically very stable in solution. The 2-*p*-chlorophenacyltelluroisochromanium ion was not fully resolved, but the optically impure enantiomorphic picrates isolated had  $[M]_D - 632^\circ$  and  $+ 575^\circ$  in acetone, and it was reasoned that the optically pure picrates would have  $[M]_D$  greater than  $750^\circ$ . The telluronium salts were much less stable optically than the sulphur and selenium analogues, racemising moderately rapidly in boiling solvents and at a measurable rate in cold moist acetone. Some mutarotation measurements were made which add further interest to the anomalous results recorded by T. M. Lowry and F. L. Gilbert.<sup>23</sup> It is to be noted that this is the first time a eutropic series of optically active compounds has been obtained and that there is a marked increase in molecular rotation in passing from sulphur through selenium to tellurium.



A useful review<sup>24</sup> is made of the stereochemistry of "square complexes". In particular the problem of the structure of bivalent platinum complexes is considered. Of all the configurations which are theoretically possible the square is the only reasonable one which is not excluded by interpretations of the phenomena of geometrical and mirror-image isomerism and spectroscopic and dipole moment measurements, or by the results of *X*-ray crystal analysis.

L. E. Marchi, W. C. Fernelius, and J. P. McReynolds<sup>25</sup> have contributed to the formal stereochemistry of compounds of elements of co-ordination number 8. From the large number of possible arrangements of eight groups round a central atom, they consider four configurations, the cube, the square Archimedean antiprism ("twisted cube"), a dodecahedron with triangular faces and with symmetry  $V_d$ , and a trigonal prism with triangular pyramids joined to the triangular faces. Isomer tables for mono- and bi-dentate groups and these configurations have been developed. Corrections to these tables were published later.<sup>26</sup>

Two of the same authors<sup>27</sup> have made a preliminary study of complex uranium oxalates. By mixing aqueous solutions of tetrapotassium uranium tetraoxalate with aqueous solutions of strychnine sulphate, various precipitates of strychnine salts were obtained, and from these the potassium

<sup>23</sup> *J.*, 1929, 2867.

<sup>24</sup> D. P. Mellor, *Chem. Reviews*, 1943, **33**, 137.

<sup>25</sup> *J. Amer. Chem. Soc.*, 1943, **65**, 329.

<sup>26</sup> *Ibid.*, 1944, **66**, 1984.

<sup>27</sup> L. E. Marchi and J. P. McReynolds, *ibid.*, 1943, **65**, 332.

salt of the acid  $H_4[U(C_2O_4)_4]$  is claimed to have been isolated in four forms, none being optically pure. The four potassium salts obtained, in order of increasing solubility (? of strychnine salt) had  $\alpha_D$  in a 2 dm. tube:  $+0.10^\circ$  ( $c$ , 0.26),  $-0.10^\circ$  ( $c$  unknown),  $+0.07^\circ$  ( $c$ , 0.47), and  $-0.05^\circ$  ( $c$ , 0.05). The experimental error was given as  $\pm 0.02^\circ$ . The solutions of the first two salts became inactive at room temperature in under one hour, the solutions of the last two salts retaining their activity for at least twelve hours. The result is taken to indicate that two alternative configurations are possible—the square Archimedean antiprism and the above dodecahedron—but considerable amplification of the experimental evidence is clearly desirable.

It will be remembered that Adams and his school<sup>28</sup> produced figures for so-called "interference values" between two atoms or groups attached one to each of the 2 and 2' positions in the diphenyl molecule. Thus, if attached to position 2 there was an atom X and to position 2' there was attached an atom Y, then, from the known (X-ray) bond lengths,  $a$  = Aromatic carbon-X, and  $b$  = Aromatic carbon-Y, and from the sum, 2.90, of two aromatic bond lengths, the interference value was calculated as  $(a + b - 2.90)$  Å. This simple calculation ignores the rather disturbing effect of any great dissimilarity in the magnitudes of  $a$  and  $b$ , but the idea served its authors a very useful purpose in that a mass of information was accumulated which related the interference value to experimental figures for comparative ease of racemisation.

The limited usefulness of the Adams method remains hardly affected by modern data for the dimensions of the diphenyl molecule. Attention may here be drawn to a precise electron-diffraction analysis of the hydrocarbon by I. L. Karle and L. O. Brockway,<sup>29</sup> who find that the 1:1' bond is  $1.54 \pm 0.03$  Å, the nuclear bonds all being  $1.39 \pm 0.04$  Å. These authors calculate that the separation of the 2 and 2' hydrogen atoms in a co-planar diphenyl molecule would be 1.84 Å. Therefore, since the nearest approach of hydrogen atoms in different molecules, as with durene, hexamethylbenzene, or methane, is 2.0 Å, energy would be required to force the diphenyl molecule into the co-planar condition.

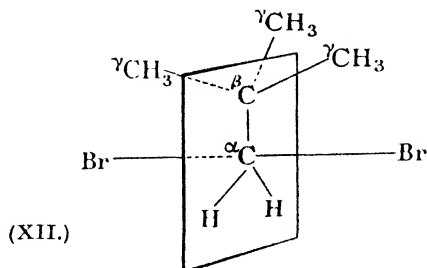
Stereochemical workers who have studied the steric factors operating in cases of dissymmetry caused by restriction of rotation will be interested in the calculations made by I. Dostrovsky, E. D. Hughes, and C. K. Ingold<sup>30</sup> of steric hindrance to the substitution reaction  $Br^- + RBr \longrightarrow RBr + Br^-$  in terms of activation energies. The reactions studied are bimolecular nucleophilic substitutions in a series of primary bromides, Me, Et, Pr<sup>n</sup>, Bu<sup>n</sup> and neopentyl, a series in which rate sequences depend less on polar factors than on the progressive building up of steric hindrance by the addition of methyl groups in the  $\beta$ -position. It is found by experiment that steric hindrance reduces the neopentyl bromide reaction rates by factors of about  $10^5$  and raises Arrhenius activation energies by about 6 kg.-cals. in

<sup>28</sup> See for example *Chem. Reviews*, 1933, 12, 261.

<sup>29</sup> *J. Amer. Chem. Soc.*, 1944, 66, 1974.

<sup>30</sup> *J.*, 1946, 173.

comparison with other primary bromides. The picture used for calculating "compression" incorporates the accepted linear transition state  $\text{Br}-\text{C}_\alpha-\text{Br}$ , that for *neopentyl* bromide (XII) being the most complicated case considered :



replacement of the methyl groups successively by hydrogen atoms illustrates all the other members of the series down to methyl bromide. The angles are either tetrahedral or follow from the geometry of the figure : the distances  $\text{Br}-\text{C}_\alpha$  are estimated by adding the covalent radius of carbon to the mean of the covalent and ionic radii of bromine : other bond lengths are taken as the sum of normal covalent radii. A shortening of the maximal van der Waals radii<sup>31</sup> when the bond makes a small or a moderate angle with the direction of a covalency is calculated and used as the distance at which two atoms come into contact as far as energy relations are concerned. From these data, the authors work out a "touching distance" (from the modified van der Waals radii) and a "model distance" (from the transition state model); the difference between these two figures is the "compression" for that particular pair of atoms. The compression so evaluated is virtually zero in all the initial states of the molecules, but in the transition state  $\text{C}_\beta-\text{Br}$  distances are compressed about 0.2 Å. and the shortest  $\text{C}_\gamma-\text{Br}$  distance by something of the order of 1 Å. : in the *neopentyl* bromide- $\text{Br}^-$  transition state there are two such  $\text{C}_\gamma-\text{Br}$  distances, accounting for the sharp rise in steric hindrance to reaction as this member of the series is reached ( $\text{Br}-\text{H}$  compressions are also evaluated).

Proceeding then, by transition state methods, the authors calculate the increment in activation energy attributable to these compressions. Assuming rigid bonding forces the results are :

*First Upper Limits to Contributions of Steric Hindrance to the Activation Energies of Br Exchange.*

	Me.	Et.	Pr <sup>β</sup> .	Bu <sup>γ</sup> .	Pr <sup>α</sup> .	Bu <sup>iso</sup> .	neoPentyl.
Energy increment, kg.-cal. ....	0.0	0.9	1.9	2.7	0.9	2.3	12.6

Small differences in these values are obtained (but amounting to nearly 1 kg.-cal. in the last case) by allowing for stretching of the  $\text{Br}-\text{C}_\alpha-\text{Br}$  bond : the values are regarded as an upper limit because no allowance is made for

<sup>31</sup> L. Pauling, "The Nature of the Chemical Bond", Cornell Press, 1940.

bending of the Br-C<sub>α</sub>-Br axis, which is expected to bring down the increment substantially.

Comparison with experiment bears witness to the essential correctness of the treatment : data of L. J. le Roux and S. Sugden<sup>32</sup> and of G. A. Elliott and S. Sugden<sup>33</sup> show that the radioactive bromine exchange reaction of three primary alkyl bromides in "90%" acetone is attended by Arrhenius activation energies as follows :

Bromide.	Pr <sup>a</sup> .	Bu <sup>a</sup> .	Bu <sup>iso</sup> .
<i>E</i> , kg.-cals. ....	~19	19.3	20.6

that is, there is a difference of about 1.3 kg.-cals. for the added β-methyl group in going from a normal to an iso-structure. The steric effect on activation energy, by calculation, varies very little when one halogen is replaced by another ; therefore the size of the substituting ion is not of the first importance and it is relevant to compare ethoxyl-bromine or iodine-bromine replacements with the above calculation :<sup>34</sup>

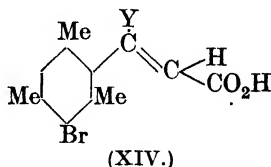
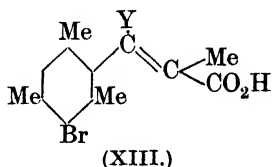
*Relative Rates and Arrhenius Activation Energies of Bimolecular Substitution of Primary Alkyl Bromides.*

	Me.	Et.	Pr <sup>a</sup> .	Bu <sup>iso</sup> .	neoPentyl.
<i>Substitution by OEt<sup>-</sup> in EtOH :</i>					
Relative rates at 55° .....	17.6	1	0.28	0.030	0.000042
<i>E</i> , kg.-cals. ....	20.0	21.0	—	22.8	26.2
<i>Substitution by I<sup>-</sup> in Me<sub>2</sub>CO :</i>					
Relative rates at 64° .....	—	1	—	—	0.000053
<i>E</i> , kg.-cals. ....	—	19	—	—	25

These observed differences in activation energy are roughly of the expected order.

Further developments of this kind of correlation will be awaited with interest.

R. Adams and his collaborators have continued their investigations of restricted rotation in arylolefinic acids, and interesting results have been obtained by varying the substituents in the olefinic grouping. All four optically active molecules of type (XIII) and (XIV), where Y is Br or Cl, show moderate optical stability, and from the rates of racemisation it is



obvious that bromine, as would be expected, has a larger steric effect than chlorine, and that the methyl group α to the carboxyl group in (XIII) induces greater stability than a hydrogen atom in the same position.<sup>35, 36</sup>

<sup>32</sup> *J.*, 1939, 1279.

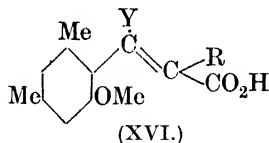
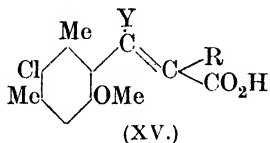
<sup>33</sup> *J.*, 1939, 1836.

<sup>34</sup> I. Dostrovsky and E. D. Hughes, *J.*, 1946, 157, 161.

<sup>35</sup> R. Adams, A. W. Anderson, and M. W. Miller, *J. Amer. Chem. Soc.*, 1941, **63**, 1589.

<sup>36</sup> R. Adams and C. W. Theobald, *ibid.*, 1943, **65**, 2383.

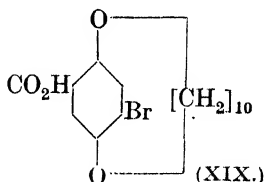
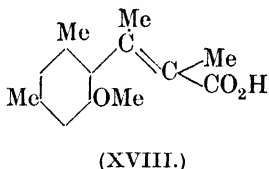
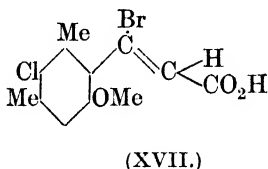
In another series of compounds<sup>37</sup> of type (XV) where Y = Cl, R = Me or Y = Cl, R = H or Y = Br, R = H, the optical isomers were relatively



unstable and those compounds in which Y = OMe, R = Me, or Y = OMe, R = H or Y = SMe, R = H, could not be resolved. Compounds in which Y = Me and R = H or Me in a series of type (XVI) were also investigated and it was deduced that the  $\beta$ -methyl group was less effective than the  $\beta$ -chlorine atom in restricting rotation.

The influence of  $\beta$ -substituents on restricted rotation in this arylacrylic acid series would therefore appear to be Br > Cl > Me, in contrast to Br > Me > Cl in the diphenyl series.<sup>38, 39</sup>

That an  $\alpha$ -methyl group in the molecule has a marked stabilising influence was illustrated by comparing the optical stability of (XVII) and (XVIII). In *n*-butyl alcohol at 22° the approximate half-life period of (XVII) was 420 minutes and that of (XVIII) was 700 minutes. Assuming that the effect of chlorine in the ring has negligible influence (demonstrated by earlier results), the greater stability of (XVIII), in spite of the fact that it contains a  $\beta$ -methyl group in place of the larger  $\beta$ -bromine in (XVII), serves to confirm this conclusion.<sup>40, 41</sup>



In amplification of a previous notice,<sup>42</sup> A. Lüttringhaus and H. Graalheer<sup>43</sup> have obtained 4-bromogentisic acid decamethylene ether (XIX) in mirror-image forms. The *l*-acid was obtained from the strychnine salt and had  $[\alpha]_D^{25} - 37.2^\circ$  in acetone, while the *d*-acid, obtained from the cinchonine salt, had  $[\alpha]_D^{25} + 37.5^\circ$ . The sodium salt of the *d*-acid was optically stable in aqueous solution at 100° over a period of three hours. The methyl ester was not racemised during saponification with methyl-alcoholic potash or when heated in toluene solution at 210° for four hours.

<sup>37</sup> R. Adams and W. J. Gross, *J. Amer. Chem. Soc.*, 1942, **64**, 1786.

<sup>38</sup> R. W. Stoughton and R. Adams, *ibid.*, 1932, **54**, 4426.

<sup>39</sup> H. C. Yuan and R. Adams, *ibid.*, p. 4434.

<sup>40</sup> R. Adams and R. S. Ludington, *ibid.*, 1945, **67**, 794.

<sup>41</sup> R. Adams and J. W. Meconey, *ibid.*, p. 798.

<sup>42</sup> *Ann. Reports*, 1941, **38**, 217.

<sup>43</sup> *Annalen*, 1941, **550**, 67.

Inhibition of rotation within the molecule arises from the constriction of the benzene nucleus within the outer ether ring, so that rotation about the O-O axis is hampered. For this type of compound the name of *ansa* (handle!) is suggested by the authors. 4-Bromogentisic acid dodecymethylene ether could not be resolved. Whether this is due to optical instability or to other causes was not decided.

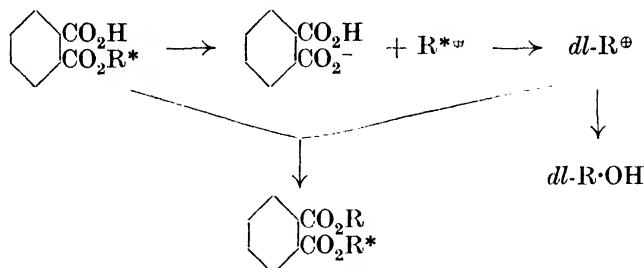
J. Kenyon and his co-workers<sup>44</sup> have continued their investigations into the properties of optically active secondary alcohols the asymmetry of which is seated in the  $\alpha$ -position: from the practical point of view of the working stereochemist the mode of hydrolysis of the esters with resolving acids or with phthalic acid of these alcohols is of prime importance. Of the alcohols studied, *p*-methoxybenzhydrol in the form of its esters most readily gives an alkyl cation ( $R'\cdot\text{CO}_2R \longrightarrow R'\cdot\text{CO}_2^- + R^+$ ), the substituted allyl and then 1-naphthylmethylcarbiny esters are next in order of stability in this sense, while phenylmethylcarbiny esters tend still less towards alkyl-oxygen fission.

Optically active 1:3-dimethylallyl hydrogen phthalate in solvolytic reactions with formic and acetic acids, hot methyl alcohol, *n*-butyl alcohol, benzyl alcohol, and phenol gives extensively racemised products (esters or ethers); if the reaction is stopped before it finishes in any of the cases the unreacted ester is shown to be largely racemised also. These results show that alkyl-oxygen fission, both during reaction and by unimolecular solvent-aided ionisation in solution, liberates momentarily a carbonium cation which can racemise. While the optically active hydrogen phthalate is stable in methyl alcohol at 31°, in nitromethane at the same temperature ionisation is facilitated and much racemisation is undergone during two months.

*p*-Methoxybenzhydrol and its esters and ethers show their tendency to alkyl-oxygen fission by ready conversion into *p*-methoxybenzhydrol chloride on treatment with cold concentrated hydrochloric acid. As might be expected, the hydrolysis of (+)-*p*-methoxybenzhydrol hydrogen phthalate results in almost complete racemisation of the alcohol even when strong aqueous alkali is used: the highest retention of optical activity is obtained when using only 2% of water in the alcohol in an attempt to employ conditions most favourable for suppressing alkyl-oxygen fission; the optically active carbinol becomes racemised even on heating alone in water. The alkyl-oxygen bond is so weak that the separation of an insoluble product (in presence of a reagent) can determine the course of a reaction: for example, when (+)-*p*-methoxybenzhydrol hydrogen phthalate is dissolved in 0.15*N*-sodium hydroxide (1 mol.), separation of an oil begins immediately, and is complete after eighteen hours, leaving only sodium phthalate in solution. The oil is *p*-methoxybenzhydrol of low dextro-rotation and

<sup>44</sup> M. P. Balfe, H. W. J. Hills, J. Kenyon, H. Phillips, and B. C. Platt, *J.*, 1942, 556; M. P. Balfe, M. A. Doughty, J. Kenyon, and R. Poplett, *J.*, 1942, 605; M. P. Balfe, E. A. W. Downer, A. A. Evans, J. Kenyon, R. Poplett, C. E. Searle, and A. L. Tárnoky, *J.*, 1946, 797.

a di-*p*-methoxybenzhydryl phthalate which contains one (+)-*p*-benzhydryl radical and one which has been racemised during migration :



Neutral ester with half optical activity.

The (+)hydrogen phthalates of 1-naphthylmethylcarbinol and phenylmethylcarbinol react with anhydrous formic acid to give the *dl*-formate. Neither hydrogen phthalate reacts with anhydrous methyl or ethyl alcohol, but on addition of water to the methyl alcohol (increasing the ionising power of the medium) 1-naphthylmethylcarbinyl hydrogen phthalate forms a racemic methyl ether. Hydrolysis of the hydrogen phthalate of optically active 1-naphthylmethylcarbinol with weak alkali (sodium carbonate) results in about 50% racemisation, but similar treatment of phenylmethylcarbinyl esters gives almost as little racemisation as is obtained on using 10*N*-sodium hydroxide.

Some other cases of alkyl-oxygen fission of alcohols and their derivatives, previously described in detail, are listed by these authors; for example D. I. Duveen and J. Kenyon<sup>45</sup> found that the (−)hydrogen phthalate of 2-furylmethylcarbinol can be hydrolysed by 10*N*-sodium hydroxide to give the optically pure alcohol, whereas hydrolysis with sodium carbonate yields a racemic alcohol. Attention is drawn to the fact that tendency towards such a mode of reaction parallels the electron-releasing power of the alkyl group.

Under appropriate conditions, similar esters with stable, optically active acids should be interesting material for studies in asymmetric transformations.

Replying to a criticism by H. I. Bernstein and E. S. Wallis<sup>46</sup> that the optical rotations on which J. Kenyon and D. P. Young<sup>47</sup> based their interpretation of the Beckmann change were rather small, A. Campbell and J. Kenyon<sup>48</sup> have provided the fresh experimental evidence in Beckmann, Lossen, and Curtius changes tabulated below :

Starting material.	Product.	Type of rearrangement.	Retention of optical activity (%).	[α] <sub>D</sub> of acetyl derivative (pure) (−168.1°).
(+)Ph·CHMe·C(:NOH)Me (2.5 g.)	(−)Acet-1-phenylethylamide (1.4 g.)	Beckmann	99.6	−167.4°
(+)Ph·CHMe·C(:NOH)·OH (3.8 g.)	(−)1-Phenylethylamine (1.8 g.)	Lossen	99.2	−166.9
(+)Ph·CHMe·CO <sub>2</sub> H (5 g.)	(−)1-Phenylethylamine (2.9 g.)	Schmidt (modified Curtius)	99.6	−165.2

<sup>45</sup> *J.*, 1936, 621. <sup>46</sup> *J. Org. Chem.*, 1942, 7, 262. <sup>47</sup> *J.*, 1941, 263. <sup>48</sup> *J.*, 1946, 25.

Coupled with the studies of C. L. Arcus and J. Kenyon of the Hofmann reaction on (+)hydratropamide, the authors conclude that the evidence points to the retention of molecular asymmetry during the migrations, and also of molecular configuration. A. E. Brodski and G. P. Mikluchin<sup>49</sup> suggest that, since in obtaining benzanilide from benzophenone oxime hydrochloride by a Beckmann change <sup>18</sup>O from water used in the final decomposition can be detected in the product, the change must be *inter-molecular*. Campbell and Kenyon point out that this could be so for the hydroxyl group without being true for the migrating group, but that no inference could be drawn from the results of a reaction using phosphorus pentachloride, since the intermediate compound must have the oxygen replaced during the reaction.

M. M. J.

M. S. L.

E. E. T.

#### 4. CARBOHYDRATES.

##### *The Chemistry of the Inositols.*

Special interest is attached to cyclic alcohols in view of the action of *meso*inositol as a growth-promoting factor in the Bios group<sup>1</sup> and because of the widespread occurrence in Nature of its hexaphosphoric ester, phytin.<sup>2</sup> Of even greater interest is the occurrence of inositol derivatives as constituents of certain bacterial lipoids<sup>3</sup> and of streptomycin.<sup>4</sup> The possibility also exists that inositol may be an intermediate in the biochemical transformation of glucose into sugars such as *d*-ribose, and into benzene derivatives.<sup>5, 6, 7</sup> Since no account has been given, within recent years, of the chemistry of the inositols and their derivatives, it has been considered opportune to compile a survey of the literature in this field.

*meso*Inositol (I),  $C_6H_{12}O_6$ , was discovered by D. Scherer<sup>8</sup> in muscle, and L. Maquenne<sup>9</sup> demonstrated that it was a cyclic hexahydroxy-alcohol. The determination of the configuration of this optically inactive compound was attended by many experimental difficulties. Reactions which could be utilised in the sugar series to determine the presence or absence of *cis*-hydroxyl groups are not specific in the cyclitol series. Recourse had to be made to an examination of the partly phosphorylated inositols and the products produced on oxidising them with potassium permanganate. By the action

<sup>49</sup> *Compt. rend. Acad. Sci. U.R.S.S.*, 1941, **32**, 558.

<sup>1</sup> F. Kögl and W. van Hasselt, *Z. physiol. Chem.*, 1936, **242**, 74.

<sup>2</sup> V. Suzuki, K. Yoshimura, and M. Takaishi, *Bull. Coll. Agric. Toyko*, 1906, **7**, 503.

<sup>3</sup> "An Introduction to Bacteriological Chemistry", C. G. Anderson, E. & S. Livingstone Ltd., Edinburgh, p. 344.

<sup>4</sup> R. L. Peck, C. E. Hoffhine, jnr., E. W. Peel, R. P. Graber, F. W. Holly, R. Mozingo, and K. Folkers, *J. Amer. Chem. Soc.*, 1946, **68**, 776.

<sup>5</sup> F. Micheel, *Annalen*, 1932, **496**, 77.

<sup>6</sup> T. Posternak, *Helv. Chim. Acta*, 1929, **12**, 1171.

<sup>7</sup> J. Needham, *Biochem. J.*, 1924, **18**, 1371; 1929, **23**, 319.

<sup>8</sup> *Annalen*, 1850, **73**, 322.

<sup>9</sup> *Ann. Chim.*, 1887, **12**, 80.

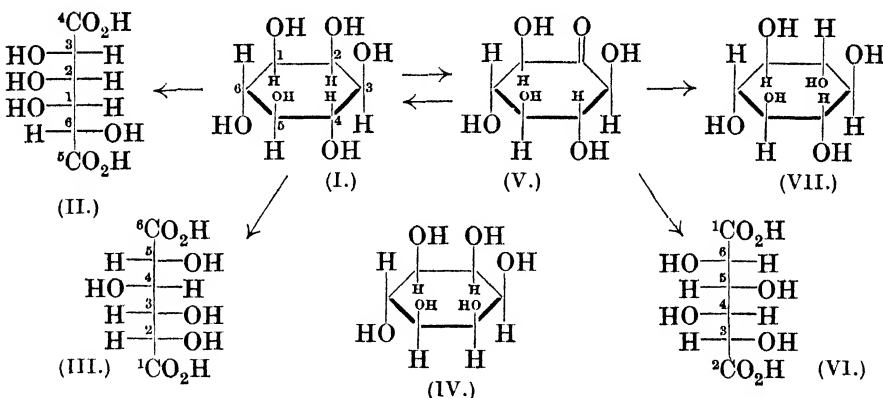


of phosphatase on phytin, an optically active tetraphosphoric ester <sup>10</sup> and an optically inactive monophosphoric ester of *mesoinositol* were obtained.<sup>11</sup>

The isolation of the latter derivative excluded formula  $\frac{1234}{56}$  whilst the isolation of the optically active derivative of *mesoinositol* showed that it could not have the configuration  $\frac{123456}{0}$ . (The numbers in the numerator or denominator

indicate the position of the hydroxyl groups above or below the plane of the ring respectively.) Oxidation with fuming nitric acid of a mixture of *meso*-inositol mono- and di-phosphates leads to the isolation of racemic and *meso*-tartaric acid. These observations exclude formula  $\frac{135}{246}$  which contains *trans*-

hydroxyl groups only and therefore would not give *mesotartaric* acid on oxidation. Furthermore, oxidation of *mesoinositol* by alkaline permanganate gives, in addition to trihydroxyglutaric acid, a mixture of tetrahydroxyadipic acids from which a racemic mixture of *d*-(II) and *l*-talomucic acid\* and a racemic mixture of *d*-(III) and *l*-saccharic acids were isolated. The only remaining configurations for *mesoinositol* consistent with this evidence are  $\frac{12345}{6}$  (IV) and  $\frac{1235}{46}$  (I).



T. Posternak <sup>12</sup> was able to decide between these two possibilities from an examination of the reactions of the oxidation product produced by the action of *Acetobacter suboxydans* on *mesoinositol*. This resulted in the formation of a ketose, inosose (V),<sup>12a</sup> which on oxidation gave a racemic mixture of *d*-(VI) and *l*-idosaccharic acid and on reduction regenerated *mesoinositol* and yielded simultaneously another substance, scyllitol <sup>13</sup> (cocosite, cocositol,

<sup>10</sup> T. Posternak, *Helv. Chim. Acta*, 1935, **18**, 1283.

<sup>11</sup> R. J. Anderson, *J. Biol. Chem.*, 1914, **18**, 441.

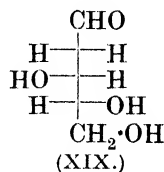
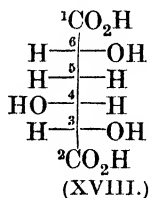
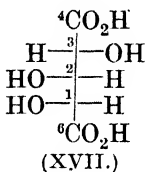
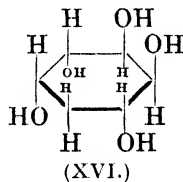
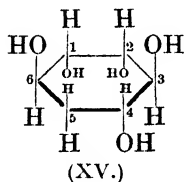
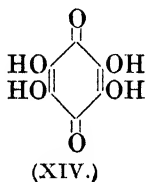
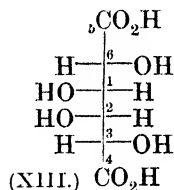
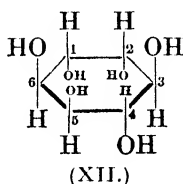
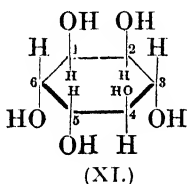
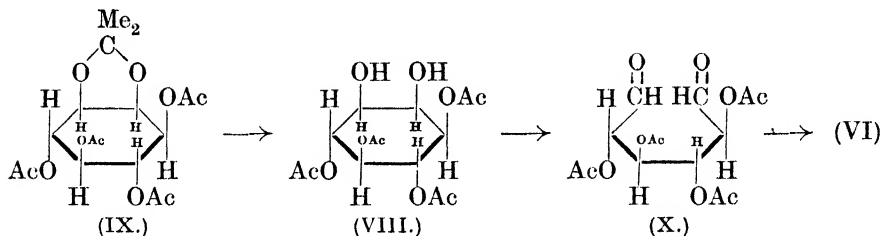
<sup>12</sup> *Helv. Chim. Acta*, 1942, **25**, 746.

<sup>12a</sup> A. J. Kluyver and A. G. J. Boezaardt, *Rec. Trav. chim.*, 1939, **58**, 956.

<sup>13</sup> G. Staedeler and F. H. Frerichs, *J. pr. Chem.*, 1858, **73**, 53; H. Müller, *J.*, 1907, 1767.

\* Initially incorrectly identified as *allomucic* acid.

quercin, quercinite), which is also a naturally occurring cyclitol. *meso*-Inositol must therefore be (I) and scyllitol which occurs in *Acanthias vulgaris* must be (VII). G. Dangschat and H. O. L. Fischer<sup>14</sup> arrived independently at the same configuration for *meso*inositol from a study of the oxidation products of its tetra-acetate (VIII), which had been prepared from the tetra-acetyl monoacetone derivative (IX) by de-acetonation.



This product (VIII) underwent oxidation with lead tetra-acetate in benzene with the formation of the dialdehyde derivative (X) identified after oxidation as a racemic mixture of the tetra-acetyl derivatives of the diethyl esters of *d*- (VI) and *l*-idosaccharic acid. This confirms formula (I) for *meso*inositol. *meso*Inositol also occurs in the form of a dimethyl ether ("dambonite"),<sup>15</sup> the constitution of which is unknown.

Only two optically active inositols are known. These are the *d*- and *l*-isomers of the cyclitol (XI) which possesses the sole configuration which can give rise to optical activity.<sup>16</sup> The relative configuration of these two isomers

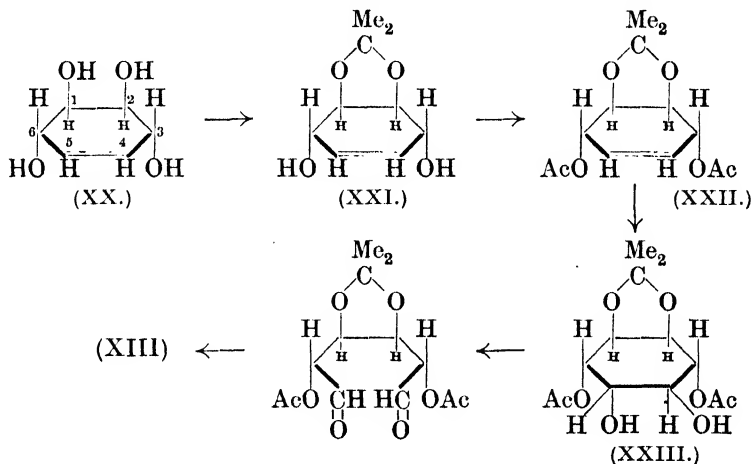
<sup>14</sup> *Naturwiss.*, 1942, **30**, 146.

<sup>15</sup> A. Girard, *Compt. rend.*, 1868, **67**, 820.

<sup>16</sup> F. Mohr, *J. pr. Chem.*, 1903, **68**, 369.

has been proved by T. Posternak,<sup>17, 18</sup> who oxidised *l*-inositol with nitric acid and isolated mucic acid (XIII) (the ring breaking between C<sub>4</sub> and C<sub>5</sub>) and *d*-saccharic acid (III) (the ring breaking between C<sub>5</sub> and C<sub>6</sub>). *l*-Inositol, therefore, has configuration (XI) and *d*-inositol is the corresponding optically active isomer (XII). These optically active cyclitols are of comparatively rare occurrence, but their corresponding monomethyl ethers (quebrachitol<sup>19</sup> and pinitol<sup>20</sup> respectively) are more widespread. The inositols can be detected by the Scherer reaction,<sup>21</sup> in which the polyhydroxy-alcohol is converted into the tetrahydroxyquinone (XIV), identified by the formation of characteristic red salts on addition of a solution of calcium or barium chloride to the oxidised material.

Deoxy-derivatives of cyclitols, like deoxy-derivatives of hexose sugars, occur in Nature. The commonest deoxycyclitol encountered is *d*-quercitol<sup>22</sup> (XV), which occurs in acorns, oak bark, etc. This substance, however, is not the optical isomer of the so-called *l*-quercitol<sup>23</sup> which occurs in the leaves of *Gymnema Sylvestra*. The configuration of *d*-quercitol was proved by T. Posternak, who examined and identified the products produced on oxidation of the cyclitol. In 1926, P. Karrer<sup>24</sup> had shown that, of twelve possibilities, only four configurations (XV and XVI and their optical isomers) were feasible for quercitol. The number of isomers is limited by the observed optical



activity of the quercitol molecule and by the observation that oxidation with nitric acid produces mucic acid (XII). In addition to this acid, *l*-arabotrihydroxyglutaric acid (XVII), and a meta-saccharic acid (XVIII), which

<sup>17</sup> A. Bouveault, *Bull. Soc. chim.*, 1894, **11**, 144.

<sup>18</sup> *Helv. Chim. Acta*, 1936, **19**, 1007.

<sup>19</sup> C. Tanret, *Compt. rend.*, 1889, **109**, 908.

<sup>20</sup> M. Berthelot, *ibid.*, 1855, **41**, 395.

<sup>21</sup> D. Scherer, *Annalen*, 1852, **81**, 375.

<sup>22</sup> C. Böttinger, *ibid.*, 1880, **202**, 282.

<sup>23</sup> F. B. Power and F. Tutin, *J.*, 1904, **85**, 624.

<sup>24</sup> *Helv. Chim. Acta*, 1926, **9**, 116.

can be converted into a deoxypentose<sup>25</sup> identical with *d*-2-deoxyxylose<sup>26</sup> (XIX), are also formed. It follows, therefore, that (XV) is the correct configuration for *d*-quercitol. Since the so-called *l*-quercitol is not the optical isomer of (XV) it may be one of the optically active forms of (XVI).

Dideoxy-derivatives of the cyclitols are known (*e.g.*, betitol<sup>26a</sup> which occurs in beet sap) but their configurations have not been ascertained. The structure of a corresponding unsaturated dideoxy-derivative—conduritol<sup>27</sup> (XX),  $C_6H_{10}O_4$ ,—was proved in 1939 by G. Dangschat and H. O. L. Fischer<sup>28</sup> by the following method. Conduritolyields a monoacetone derivative which is not oxidised by lead tetra-acetate. It therefore has structure (XXI) in which the hydroxyl groups on  $C_1$  and  $C_2$ , which have reacted with the acetone, are *cis*- to one another, the configurations of the remaining hydroxyl groups being unknown. This product after acetylation (XXII) is oxidised by neutral permanganate to a dihydroxy-compound (XXIII) which on further oxidation, first with lead tetra-acetate and then with peracetic acid, yields a diacetyl acetone derivative of mucic acid (XIII), thus proving that the hydroxyl groups on  $C_3$  and  $C_6$  are *trans*- to those on  $C_2$  and  $C_1$  respectively.

The structures of the two carboxylic acid derivatives of the cyclitols, shikimic and quinic acids, have also been investigated by G. Dangschat and H. O. L. Fischer.<sup>24</sup> Shikimic acid (XXIV) which occurs in *Illicium verum* and *I. religiosum* analyses as  $C_7H_{10}O_5$ . It contains one carboxyl group, three hydroxyl groups, and one double bond. Oxidation of the methyl ester with periodic acid yields one mol. of formic acid (thus proving the presence of hydroxyl groups on each of three contiguous carbon atoms) and a dialdehyde (XXV) which, on further oxidation with perpropionic acid forms, after hydrolysis of the ester grouping, *trans*-aconitic acid (XXVI). Shikimic acid, on hydrogenation, is converted into a saturated dihydro-derivative,<sup>29a</sup> which furnishes an optically active lactone, unaffected by periodic acid. Models show that this optically active product possesses structure (XXVII) in which the hydroxyl and carboxyl groups engaged in lactone formation are on the same side of the ring and that the remaining hydroxyl groups must be *trans*- to one another if an optically active lactone is to be produced. The configuration of the hydroxyl groups and position of the double bond was determined by converting methyl shikimate into its acetone derivative (XXVIII), the acetyl derivative of which on permanganate oxidation gave the dihydroxy-compound (XXIX). This material on further oxidation, first with periodic acid and then with bromine in acetic acid, yielded the aldehydo-acid (XXX), converted by reduction into the known *d*-2-deoxygluconic acid (XXXI) thus

<sup>25</sup> H. Kiliani and C. Scheibler, *Ber.*, 1889, **22**, 517.

<sup>26</sup> P. A. Levene and T. Mori, *J. Biol. Chem.*, 1929, **83**, 803; T. Posternak, *Helv. Chim. Acta*, 1932, **15**, 949.

<sup>26a</sup> E. von Lippman, *Ber.*, 1901, **34**, 1159.

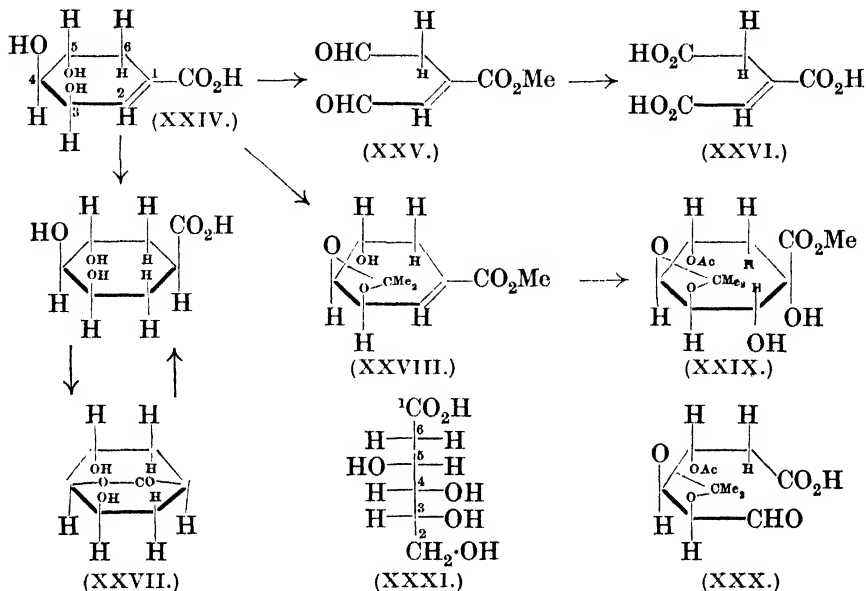
<sup>27</sup> M. Kubler, *Arch. Pharm.*, 1908, **246**, 623, 645.

<sup>28</sup> *Naturwiss.*, 1939, **27**, 756.

<sup>29</sup> *Helv. Chim. Acta*, 1934, **17**, 1200; 1935, **18**, 1204, 1206; 1937, **20**, 705.

<sup>29a</sup> K. Freudenberg, H. Meisenheimer, J. T. Lane, and E. Plankenhorn, *Annalen*, 1940, **543**, 162.

giving the exact stereochemical configuration of the hydroxyl groups in the molecule and proving shikimic acid to be (XXIV).



The hydrated form of (XXIV) is quinic acid (XXXII) which has been investigated by the same authors<sup>30</sup> and by P. Karrer, R. Widmer, and P. Riso.<sup>30a</sup> Quinic acid occurs in cinchona bark, coffee beans, etc.; it forms part of the molecule of chlorogenic acid<sup>31</sup> (XXXIII). The major part of the structure of quinic acid is proved by the conversion of its derivative, 3-acetyl 4:5-methylene quinic acid amide (XXXIV) into the nitrile of 3-acetyl 4:5-methylene shikimic acid (XXXV). The acetone derivative of quinic acid amide (XXXVI) on oxidation with periodic acid yields one mol. of formic acid and a product which, on hydrolysis after bromine oxidation, gives citric acid. It follows, therefore, that the hydroxyl group, which is removed in the conversion of the quinic acid derivative (XXXIV) into a derivative of shikimic acid (XXXV), is attached to the same carbon atom as the carboxyl group. This carboxyl group is on the same side of the ring as the hydroxyl grouping on C<sub>5</sub>,<sup>31</sup> since quinic acid will form a furanolactone while quinic acid 5-methyl ether will not.

T. Posternak<sup>32</sup> has proved that mytilitol, a cyclitol which occurs in the mussel, is (XXXVII) since it can be synthesised, together with *isomytilitol* (XXXVIIa), by the action of methylmagnesium iodide on the penta-acetyl derivative of the keto-inositol (V).<sup>12a</sup>

*meso*Inositol has been synthesised by H. Wieland and R. S. Wishart<sup>33</sup>

<sup>30</sup> *Helv. Chim. Acta*, 1934, **17**, 1197; *Ber.*, 1921, **44**, 775; 1932, **65**, 1009.

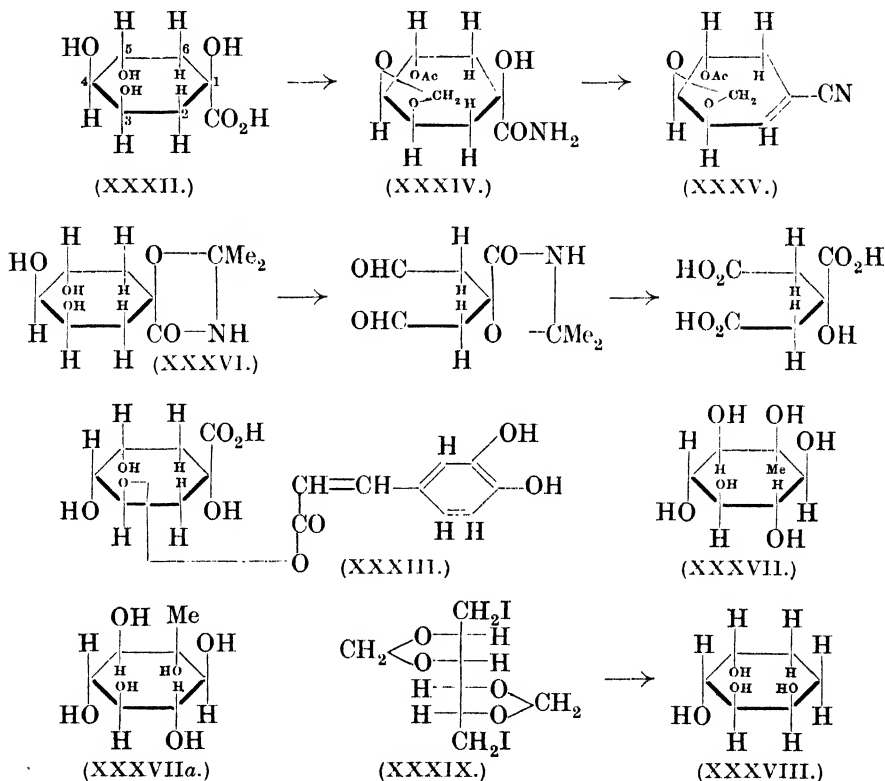
<sup>30a</sup> *Helv. Chim. Acta*, 1925, **8**, 195.

<sup>31</sup> *Ber.*, 1932, **55**, 1037.

<sup>32</sup> *Helv. Chim. Acta*, 1944, **27**, 457.

<sup>33</sup> *Ber.*, 1914, **47**, 2082.

by hydrogenation of hexahydroxybenzene. Other attempts to synthesise inositol have resulted in the formation of derivatives isomeric with betitol. F. Micheel synthesised<sup>34</sup> (XXXVIII) by the action of silver on the 1 : 6-di-iodo dimethylene mannitol (XXXIX). Y. Hamamura<sup>35</sup> used a similar method to prepare the same product. Attempts to prepare inositol from 2 : 3 : 4 : 5-tetra-acetyl mucyl dialdehyde<sup>36</sup> and from 2 : 3 : 4 : 5-tetra-acetyl mucyl dichloride<sup>37</sup> were unsuccessful.



### Hexomethyloses and the Deoxyhexomethyloses.

During the last few years considerable effort has been devoted to problems connected with the determination of the structures of the less common sugars which are found in the cardiac glycosides. These sugars are digitalose, digitoxose, oleandrose, cymarose, and sarmentose.<sup>38</sup>

Digitalose (XL) was first obtained as a syrup from *Digitalinum verum*

<sup>34</sup> *Annalen*, 1932, **496**, 77.

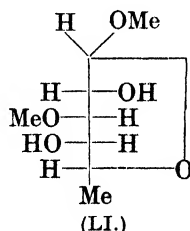
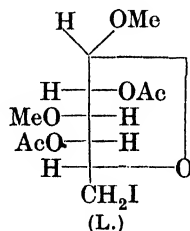
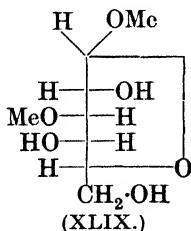
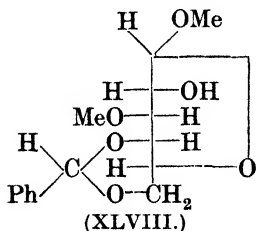
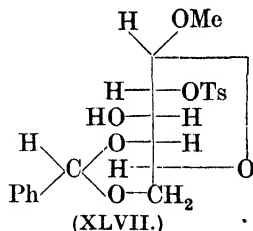
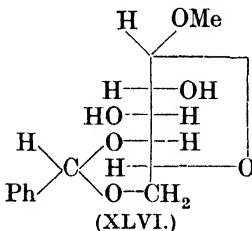
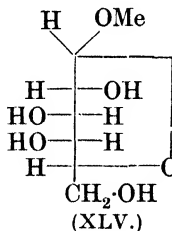
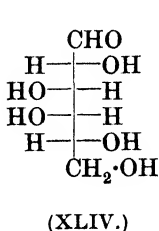
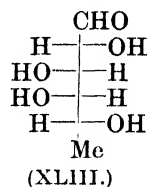
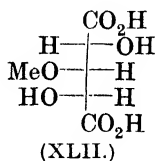
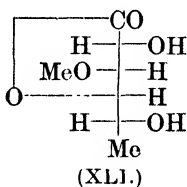
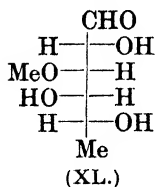
<sup>35</sup> *Proc. Imp. Acad. Tokyo*, 1934, **10**, 459.

<sup>36</sup> F. Kögl and M. Uenzelmann, *Diss. Uenzelmann*, Göttingen, 1931.

<sup>37</sup> O. Diels and F. Löffund, *Ber.*, 1914, **47**, 2351, 2827; J. Müller, *ibid.*, p. 2654.

<sup>38</sup> W. E. Bouman, *Pharm. Tijds. Nederl. Indie*, 1941, **18**, 39—177.

by H. Kiliani,<sup>39</sup> who isolated from it a crystalline osazone and a crystalline lactone,  $C_7H_{12}O_5$ . The lactone (XLI) on oxidation with silver oxide gave acetic acid, whilst nitric acid oxidation yielded a dihydroxymethoxyglutaric acid (XLII). These observations indicated that digitalose was a methoxy-derivative of a hexomethylse (XLIII).<sup>40</sup> The configuration of (XLII) was proved by the observation of O. T. Schmidt and H. Zeiser<sup>41</sup> that on complete methylation it gave *l-arabotrimethoxyglutaric acid*. Since digitalose yields an osazone without loss of methoxyl it possesses a hydroxyl group on  $C_2$ . The rate of mutarotation of (XLI) indicates that it is a furano-lactone, and the sugar (XL) therefore possesses a hydroxyl group on  $C_4$  whilst the isolation of the monomethyl *l-arabotrihydroxyglutaric acid* (XLII) after oxidation of (XL) with nitric acid, shows that the hydroxyl group on  $C_5$  is unsubstituted. The exact structure of digitalose was proved in 1943 by O. T. Schmidt, W. Mayer, and A. Distelmair,<sup>42</sup> who converted methyl digitaloside into its fully methylated glycoside which on hydrolysis gave 2 : 3 : 4-trimethyl *d*-fucose (*d*-galactomethylse). The isolation of this substance proves that the configuration of the hydroxyl groups is that of *d*-galactose (XLIV). Digitalose is therefore 3-methyl *d*-fucose (XL).



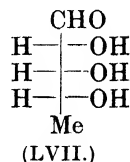
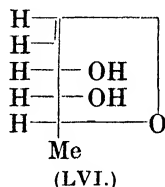
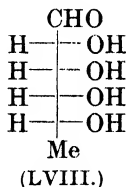
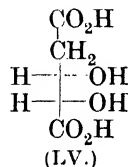
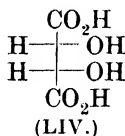
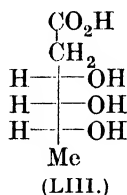
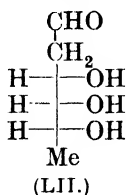
<sup>39</sup> H. Kiliani, *Ber.*, 1898, **31**, 2454.

<sup>40</sup> *Idem, ibid.*, 1905, **38**, 3621; 1922, **55**, 92; 1931, **64**, 2027.

<sup>41</sup> *Ber.*, 1934, **67**, 2127.

<sup>42</sup> *Naturwiss.*, 1943, **31**, 247; *Annalen*, 1943, **555**, 26.

The sugar was synthesised by F. Reber and T. Reichstein,<sup>43</sup> who converted methyl-*d*-galactoside (XLV) into its 4 : 6-benzylidene derivative (XLVI) and thence into its 2-*p*-toluenesulphonate (XLVII). Methylation of this with silver oxide and methyl iodide gave the corresponding 3-methyl derivative, which was converted into 4 : 6-benzylidene 3-methyl methyl-*d*-galactoside (XLVIII) after removal of the *p*-toluenesulphonyl group with sodium amalgam. This on hydrogenation with Raney nickel as catalyst gave 3-methyl methyl-*d*-galactoside (XLIX) which was converted into the digitalopyranoside (LI) by way of 6-triphenylmethyl methyl-*d*-galactopyranoside, 2 : 4-diacetyl 3-methyl methyl-*d*-galactopyranoside, and 6-iodo 2 : 4-diacetyl 3-methyl methyl-*d*-fucopyranoside (L). The identity of the synthetic digitalose (XL) was confirmed by converting it into the well-characterised crystalline lactone (XLI).



Digitoxose (LII),  $\text{C}_6\text{H}_{12}\text{O}_4$ , is a component of the glycosides digitoxin, gitoxin, and digoxin, from which it can be obtained after hydrolysis by dilute acid. It was first isolated in 1896 by H. Kiliani,<sup>44</sup> who observed that it did not form an osazone—though an aldehyde group was present since it would react with hydrogen cyanide—and that after oxidation it yielded digitoxonic acid (LIII) which contained the same number of carbon atoms. Oxidation of the sugar with silver oxide gave acetic acid, whilst oxidation with nitric acid produced *mesotartaric* acid (LIV) and 1 : 2-dihydroxyglutaric acid (LV). These observations indicate that digitoxose is a 2 : 6-dideoxyhexose and that the configuration of the hydroxyl groups is either that of ribose or arabinose. A decision between these two possibilities was made from an examination of digitoxal (LVI), which had been prepared by heating the sterol derivative, digitoxin.<sup>45</sup> This compound (LVI), which is an unsaturated derivative of the glucal type, yields on ozonisation a pento-methyllose (LVII) identified as *d*-ribomethyllose.<sup>46</sup> Digitoxose is, therefore, a derivative of *d*-allose (or

<sup>43</sup> *Helv. Chim. Acta*, 1946, **29**, 343.

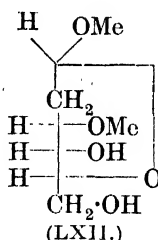
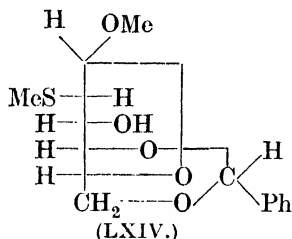
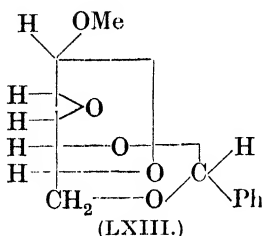
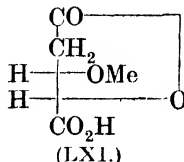
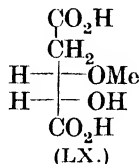
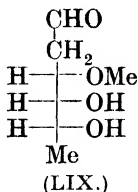
<sup>44</sup> H. Kiliani, *Arch. Pharm.*, 1896, **234**, 438; *Ber.*, 1913, **46**, 667.

<sup>45</sup> A. Windaus and G. Stein, *Ber.*, 1928, **61**, 2436.

<sup>46</sup> F. Mischeel, *Ber.*, 1930, **63**, 347.



*d*-altrose). This was confirmed by its synthesis from *d*-allomethylose (LVIII)<sup>47</sup> (obtained from *l*-rhamnose by the method of P. A. Levene and J. Compton).<sup>48</sup> The sugar was converted into acetobromo-*d*-allomethylose, and then into digitoxal diacetate which on hydrolysis with *N*-sulphuric acid gave (LII). Digitoxose has been characterised as its benziminazole derivative.<sup>49</sup>



From a number of cardiac glycosides the sugar, cymarose (LIX),  $\text{C}_7\text{H}_{14}\text{O}_4$ , can be isolated after acid hydrolysis. A. Windaus and L. Hermanns,<sup>50</sup> who first prepared cymarose in the crystalline state, showed that on oxidation with silver oxide it gave acetic acid and that with phenylhydrazine it gave a phenylhydrazone but no phenylosazone. These observations, and the fact that the sugar gave the colour reactions of digitoxose, led them to suggest that cymarose was a methyl ether of digitoxose (LII). Cymarose on oxidation with nitric acid gives a hydroxymethoxyglutaric acid (LX) which will form a furano-lactone (LXI); it is inferred then that there is a hydroxyl group adjacent to one of the carboxyl groups in (LX).<sup>51</sup> The proof of the configuration of the hydroxyl and the methoxyl groups in cymarose is furnished by the observation that the fully methylated derivative of digitoxose is identical with that of cymarose. Final proof of the structure of cymarose has been given by its synthesis from 3-methyl 2-deoxy- $\alpha$ -methyl-*d*-allopyranoside (LXII).<sup>52, 53</sup> This compound was prepared by a novel route from 4 : 6-benzylidene 2 : 3-anhydromethyl-*d*-alloside (LXIII) which was converted into the 2-methylthio-derivative of 4 : 6-benzylidene  $\alpha$ -methyl-*d*-altropyranoside (LXIV) by reaction with sodium thiomethoxide. By boiling the corresponding 3-methyl ether with Raney nickel, sulphur and

<sup>47</sup> B. Iselin and T. Reichstein, *Helv. Chim. Acta*, 1944, **27**, 1203.

<sup>48</sup> *J. Biol. Chem.*, 1936, **116**, 176.

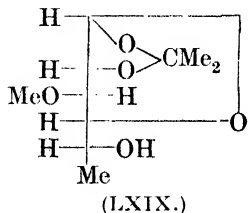
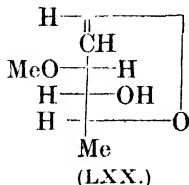
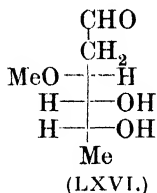
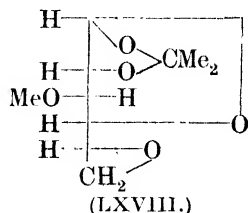
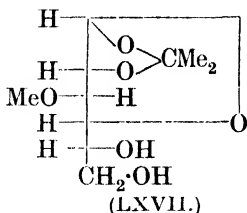
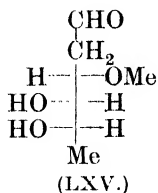
<sup>49</sup> R. J. Dimler and K. P. Link, *ibid.*, 1943, **150**, 345. <sup>50</sup> *Ber.*, 1915, **48**, 979.

<sup>51</sup> R. C. Elderfield, *J. Biol. Chem.*, 1935, **111**, 527.

<sup>52</sup> C. A. Grob and D. A. Prins, *Helv. Chim. Acta*, 1945, **28**, 840.

<sup>53</sup> R. Jeanloz, D. A. Prins, and T. Reichstein, *ibid.*, 1946, **29**, 371.

benzaldehyde<sup>54, 55</sup> were eliminated and 3-methyl-2-deoxy- $\alpha$ -methyl-*d*-allopyranoside resulted. This was then converted into (LIX) by way of the 6-*p*-toluenesulphonate of (LXII) and the 6-iodo-derivative of (LIX).



Oleandrose  $\text{C}_7\text{H}_{14}\text{O}_4$  (LXV) was isolated in the crystalline form by W. Neumann<sup>56</sup> from the glycoside oleandrin, which is present in the leaves of the oleander (*Nerium oleander*). Oleandrose shows a positive Keller-Kiliani reaction, forms a phenylhydrazone and not a phenylosazone, and is an aldehyde since on oxidation it yields an acid containing the same number of carbon atoms. It contains one methoxyl group, and on oxidation yields acetic acid and *l*(+)-methoxysuccinic acid; it is therefore a monomethyl dideoxyhexose. The optical isomer of *l*-oleandrose, namely, 3-methyl 2-deoxy-*d*-quinovose (LXVI), has been synthesised and its structure proved in the following manner.<sup>57</sup> 3-Methyl 1 : 2-isopropylidene-*d*-glucose (LXVII) was converted into the 5 : 6-anhydro-derivative (LXVIII) by alkaline hydrolysis of the 6-*p*-toluenesulphonate of (LXVII). The anhydro-ring was opened by catalytic reduction at 100 atmospheres' pressure, and the resultant quinovose derivative (LXIX) hydrolysed to 3-methyl quinovose which was converted through its acetobromo-derivative into 3-methyl *d*-quinoval (LXX), hydrolysis of which with *N*-sulphuric acid yielded *d*-oleandrose (LXVI), the optical isomer of the naturally occurring compound (LXV). *d*-Oleandrose is not identical with the naturally occurring diginose, which appears to be the 3-methyl ether either of a derivative of 2-deoxy-*d*-fucose or of 2-deoxy-*l*-gulomethylose.<sup>57a, 57b</sup> The structure of sarmentose is unknown.

<sup>54</sup> M. L. Wolfrom and J. V. Karabinos, *J. Amer. Chem. Soc.*, 1944, **66**, 909.

<sup>55</sup> D. A. Prins, *Helv. Chim. Acta*, 1946, **29**, 378.

<sup>56</sup> *Ber.*, 1937, **70**, 1547.

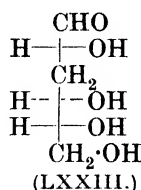
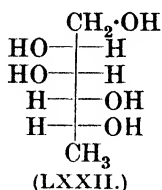
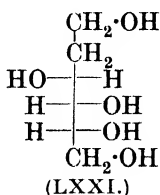
<sup>57</sup> E. Vischer and T. Reichstein, *Helv. Chim. Acta*, 1944, **27**, 1332.

<sup>57a</sup> C. W. Shoppee and T. Reichstein, *ibid.*, 1942, **25**, 1611.

<sup>57b</sup> *Idem*, *ibid.*, p. 975.

<sup>57c</sup> A. C. Mackly and T. Reichstein, *ibid.*, 1947, **30**, 496.

The synthesis of *l*-idomethylose from 4 : 6-benzylidene methylgalactoside and from 1 : 2-*isopropylidene d*-glucofuranose,<sup>58</sup> and of 2-deoxy-*l*-fucose,<sup>58a</sup> 2-deoxy-*d*-allose,<sup>58b</sup> and 2-deoxy-*l*-rhamnose<sup>58c</sup> has also been reported.



The configuration of the hydroxyl groups in three of the sugars (digitalose, digitoxose, and cymarose) is unusual in that they are derivatives of *d*-fucose and *d*-allose (or *d*-altrose), sugars which occur but rarely in nature. *d*-Fucose has been detected in the glycosides convolvulin<sup>59</sup> and jalopin<sup>60</sup> only, whilst the *d*-allose configuration is present in sedoheptulose and volemitol which occur in plants of the family *Crassulaceae*.

2-Deoxysorbitol (LXXI) has been isolated from the mixture of products produced on electro-reduction of *d*-glucose; *d*-rhamnitol<sup>61</sup> (LXXII), *l*-gulomethylitol, allitol, a branched chain pentitol, and 2-deoxyallitol(?) were also detected. These results are in harmony with an enolic mechanism of sugar interconversion under reducing conditions. In a further publication the isolation of small amounts of *dl*-sorbitol<sup>62</sup> from the electrolytic reduction of *d*-glucose was announced. This formation of *l*-sorbitol requires the inversion of the hydroxyl groups on C<sub>3</sub> and C<sub>4</sub> of the *d*-glucose molecule. The unusual sugar 3-deoxy-*d*-glucose (allose) (LXXIII) has been prepared by D. A. Prins<sup>63</sup> by ring scission of 4 : 6-benzylidene 2 : 3-anhydro-methyl-*d*-allopyranoside with hydrogen and Raney nickel under pressure. 3-Deoxy-*d*-mannose has also been prepared.<sup>63a</sup>

### *The Chromatographic Separation of Carbohydrates and Related Compounds.*

Since the discovery by W. S. Reich<sup>64</sup> that a mixture of the *p*-phenylazo-benzoates of *d*-fructose and *d*-glucose yielded two visible bands on a chromatogram developed on silica gel or alumina, the use of this technique has been extended. It has been utilised, for example, by G. H. Coleman and others in the separation of pentose and hexose sugars and of mono- and di-saccharides

<sup>58</sup> A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, 1946, **29**, 137, 152.

<sup>58a</sup> B. Iselin and T. Reichstein, *ibid.*, 1944, **27**, 1200.

<sup>58b</sup> R. Jeanloz, D. A. Prins, and T. Reichstein, *ibid.*, 1946, **29**, 371.

<sup>58c</sup> B. Iselin and T. Reichstein, *ibid.*, 1944, **27**, 1146.

<sup>59</sup> E. Votoček, *Ber.*, 1904, **37**, 3861, 4615.

<sup>60</sup> E. Votoček and J. Kastner, *Z. Zuck. Ind. Böhm.*, 1907, **31**, 307.

<sup>61</sup> M. L. Wolf from, M. Konigsberg, F. B. Moody, and R. M. Goepf, jr., *J. Amer. Chem. Soc.*, 1946, **68**, 122, 578, 1443.

<sup>62</sup> M. L. Wolf from, B. W. Lew, R. A. Hales, and R. M. Goepf, jr., *ibid.*, p. 2342.

<sup>63</sup> *Helv. Chim. Acta*, 1946, **29**, 1.

<sup>63a</sup> D. A. Prins, *ibid.*, p. 1061.

<sup>64</sup> *Compt. rend.*, 1939, **208**, 589, 748; *Biochem. J.*, 1939, **83**, 1000.

on magnesite, dicalite, or silica gel. In a similar manner the same workers have separated the products resulting on methanolysis of heptamethyl methyl-cellobioside, -maltoside, -gentiobioside, and -melibioside.<sup>65</sup> The method of W. S. Reich has the advantage that the separation of the sugar derivatives can be followed visually, but it suffers from the disadvantage that the yields of *p*-phenylazobenzoyl derivative are not quantitative and that mixtures of isomers may be produced.

M. L. Wolfrom and his co-workers<sup>66</sup> in a series of publications describe methods for the separation of sugars and sugar alcohols and of their acetyl derivatives on columns of "Magnesol" and "Celite". The brush or streak technique, as practised by Zechmeister,<sup>67</sup> was employed to observe the separation, the indicator used being either alkaline potassium permanganate, 2 : 6-dichlorophenol-indophenol, or acid-base indicators. The separation of acetylated derivatives of the sugars was unsatisfactory in that considerable losses may occur in the preparation of the acetyl derivatives and quantitative recovery of the sugars is then not possible. The separation of the sugars and of the sugar alcohols<sup>68</sup> was achieved by chromatography on "Silene E.F." plus synthetic hydrated calcium acid silicate using 90% dioxan or 90% ethanol as solvent and aqueous isopropanol as developer. The yields of sugars isolated varied from 78 to 90%. A useful table showing the relative strength of adsorption of various sugars and alcohols under these conditions is given by the authors.<sup>69</sup>

The purification of sugars and their derivatives by chromatography has been employed by several groups of workers. W. W. Binkley and M. L. Wolfrom<sup>70</sup> use the methods they have developed to prove that the condensation of 1 : 3 : 4 : 6-tetra-acetyl *d*-fructofuranose and 2 : 3 : 4 : 6-tetra-acetyl *d*-glucopyranose with phosphoric oxide yields an isosucrose derivative;<sup>71</sup> no trace of sucrose octa-acetate could be detected, thus confirming the earlier work of (Sir) J. C. Irvine and E. T. Stiller. The separation of synthetic octa-acetyl cellobiose prepared by the condensation of acetobromoglucose with the sodium derivative of 1 : 2 : 3 : 6-tetra-acetyl glucose, and of octa-acetyl gentiobiose prepared from acetobromoglucose and the sodium derivative of 1 : 2 : 3 : 4-tetra-acetyl glucose, from impurities was also achieved by chromatography, in this case on silica gel.<sup>72</sup> H. R. Bullinger and D. A. Prins<sup>73</sup> used chromatography to isolate a pure specimen of 4 : 6-hexahydrobenzylidene 3-deoxy- $\alpha$ -methyl-*d*-glucoside. The method

<sup>65</sup> *J. Amer. Chem. Soc.*, 1942, **64**, 1501; 1943, **65**, 1588; 1945, **67**, 381; cf. K. Myrbäck and C. O. Tamm, *Svensk. Kem. Tid.*, 1941, **53**, 441; K. Freudenberg and H. Boppel, *Ber.*, 1940, **73**, 609.

<sup>66</sup> *J. Amer. Chem. Soc.*, 1945, **67**, 527.

<sup>67</sup> *Bull. Soc. Chim. biol.*, 1936, **18**, 1885.

<sup>68</sup> B. W. Lew, M. L. Wolfrom, and R. M. Goepp, jnr., *J. Amer. Chem. Soc.*, 1945, **67**, 1865.

<sup>69</sup> L. W. Georges, R. S. Bower, and M. L. Wolfrom, *ibid.*, 1946, **68**, 2169.

<sup>70</sup> *Ibid.*, pp. 2171, 1720.

<sup>71</sup> *Ibid.*, 1932, **54**, 1079.

<sup>72</sup> V. E. Gilbert, F. Smith, and M. Stacey, *J.*, 1946, 622.

<sup>73</sup> *Helv. Chim. Acta*, 1945, **28**, 465.

used by A. H. Gordon, A. J. P. Martin, and R. L. M. Synge<sup>74</sup> for the separation of amino-acids on moist filter paper by allowing a suitable solvent, which has previously been saturated with water, to flow over the paper in a closed container, has been used for the qualitative separation and identification of sugars on the micro-scale.<sup>75</sup> Ammoniacal silver nitrate, reduced by the sugars to silver, was used to reveal their position on the chromatograms.

The quantitative separation of the methyl ethers of the sugars has been investigated. D. J. Bell<sup>76</sup> used silica gel to separate practically quantitatively as little as 50 mg. of tetramethyl *d*-glucose from as much as 10 g. of 2 : 3 : 6-trimethyl *d*-glucose. Separation is achieved by developing with chloroform or with *n*-butanol and chloroform if dimethyl glucose is to be separated from 2 : 3 : 6-trimethyl *d*-glucose; the separation is followed by testing portions of the column for the presence of sugars by the Molisch reaction. The procedure is analogous to that used for the separation of acetamido-acids.<sup>77</sup> The second method of separation is dependent upon the observation that the methylated sugars fluoresce in ultra-violet light.<sup>78</sup> The mixture of methylated sugars is dissolved in benzene and the solution passed through a column of activated alumina (non-fluorescent). Benzene is passed down the column and the development of the chromatogram observed in ultra-violet light. The recovery of material is 95–105% on quantities as small as 20 mg. J. K. N. Jones<sup>79</sup> dissolved the sugar glycosides in light petroleum and passed the solution down a column of activated alumina. Tetramethyl methyl-*d*-glucosides, which have the lowest adsorptive strength, were separated by washing them through the column with light petroleum. The progress of the elution was followed by testing the eluate for sugars by the Molisch reaction and by evaporating portions of the solvent and weighing the residue. The efficiency of the separation was determined from the refractive index and methoxyl content of the residual syrup. The recovery of tetramethyl methylglucoside was 94% on weights of 150 mg.

A partial separation of trimethyl methyl-*l*-arabofuranoside could be effected from a mixture of this sugar and 2 : 3 : 4-trimethyl methyl-*d*-xyloside. The fractionation of a methylated polysaccharide by chromatography on alumina was also achieved.

#### *Oxidation of $\alpha$ -Glycol Groups.*

(Continued from *Annual Reports*, 1943, **40**, 115.)

Lead tetra-acetate and periodic acid, reagents diagnostic for the  $\alpha$ -glycol group, continue to be used extensively in the determination of the structure of sugars and their derivatives. It has been suggested that lead tetra-acetate causes the splitting of  $\alpha$ -glycols because of formation of free acetate radicals

<sup>74</sup> *Biochem. J.*, 1943, **37**, Proc. XIII; 1944, **38**, 224.

<sup>75</sup> S. M. Partridge, *Nature*, 1946, **158**, 270.

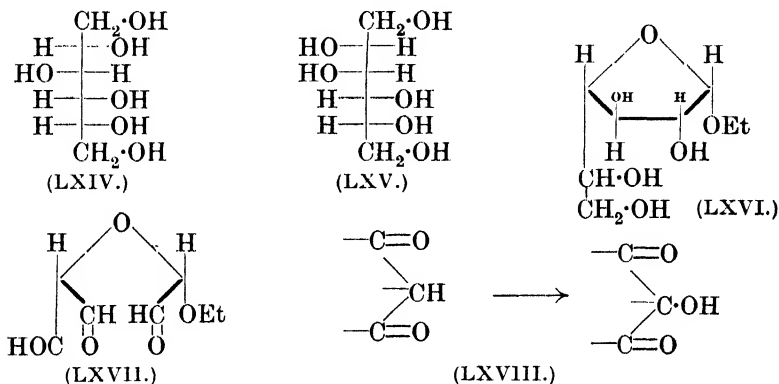
<sup>76</sup> D. J. Bell, *J.*, 1944, 473.

<sup>77</sup> A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, 1941, **35**, 1388; 1943, **37**, 79.

<sup>78</sup> E. J. Norberg, I. Auerbach, and R. M. Hixon, *J. Amer. Chem. Soc.*, 1945, **67**, 342.

<sup>79</sup> *J.*, 1944, 333.

which dehydrogenate the alcohol grouping.<sup>80</sup> R. C. Hockett, in a series of papers, has examined the reaction between lead tetra-acetate and a large number of sugar derivatives. The following rules are postulated: (a) lead tetra-acetate will oxidise a vicinal triol with consumption of 2 mols. of reagent and formation of one mol. of formic acid; the acid may then undergo oxidation with the reduction of further quantities of lead tetra-acetate. (b) *cis*-Hydroxyl groups are more rapidly oxidised than *trans*-hydroxyl groups. (c)  $\alpha$ -Hydroxy-aldehydes are attacked but slowly unless furano- or pyranoring formation is possible owing to the presence of a suitably placed hydroxyl group; oxidation is then much more rapid because of the formation of a hemiacetal which simulates an  $\alpha\beta$ -glycol. Using these rules the structures



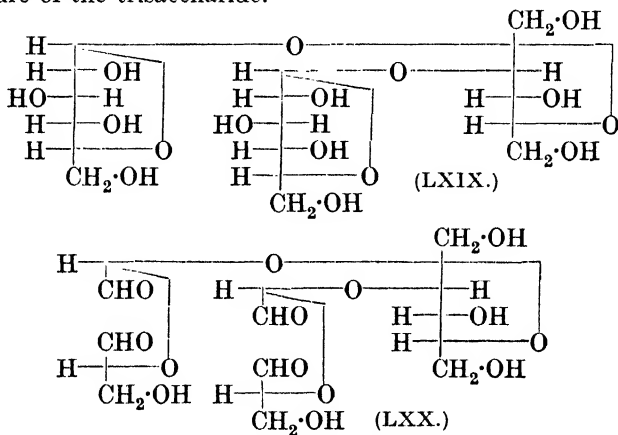
of the di- and tri-benzoates of *d*-sorbitol (LXIV) and *d*-mannitol (LXV) were proved. Styracitol was similarly shown to be 1:5-anhydro-*d*-mannitol. Methyl- $\alpha$ -*d*-mannofuranoside reacts rapidly with lead tetra-acetate, consuming one mol. of the reagent. No formaldehyde is detectable at this stage, proving that it is the two *cis*-hydroxyl groups that are oxidised. Further consumption of the oxidant proceeds much more slowly owing to hemiacetal formation between the primary alcohol grouping on  $\text{C}_6$  and the aldehyde residue on  $\text{C}_3$ . The hydroxyl groups attached to the ring in  $\alpha$ -ethyl-*d*-galactofuranoside (LXVI) are *trans*- and are therefore not attacked more rapidly than the *exocyclic* hydroxyl groups. Two mols. of lead tetra-acetate are consumed in this oxidation with production of formaldehyde. Oxidation of hexofuranosides with periodic acid or its salts proceeds in a similar manner. This reaction, however, is complicated by further oxidation of the trialdehyde (LXVII).<sup>81</sup> It has been shown that the periodate ion will oxidise hydrogen to hydroxyl when it is attached to a carbon atom situated between two carbonyl groups (LXVIII); the product may then undergo further oxidation. This observation explains why substances of the malonic acid type are oxidised by the periodate ion and why derivatives of the hexo-

<sup>80</sup> A. H. Waters, *Nature*, 1946, **158**, 380.

<sup>81</sup> R. C. Hockett, (Miss) M. T. Dienes, and H. E. Ramsden, *J. Amer. Chem. Soc.*, 1943, **65**, 1474; R. C. Hockett and H. G. Fletcher, jnr., *ibid.*, 1944, **66**, 467, 469.

furanosides, methyl-*d*-galacturonoside and bornyl-*d*-glucuronoside consume more than the expected two mols. of periodate.<sup>82, 82a</sup>

The products formed on reaction of the periodate ion with sugars are dependent upon the pH and temperature of the solution. If the temperature is raised and the pH becomes more alkaline than 5, quantities of carbon dioxide and formic acid may be produced.<sup>83</sup> At a lower pH and temperature formic acid and formaldehyde are the sole detectable products after oxidation of aldoses, but ketoses may undergo oxidation with the production of up to 0.8 mol. of carbon dioxide.<sup>84</sup> Sucrose has been shown to behave in the normal manner on oxidation with periodic acid with formation of the expected tetra-aldehyde, which can be oxidised to a tetracarboxylic acid.<sup>85</sup> This acid on hydrolysis yields glyceric, glyoxylic, and hydroxypyruvic acids. Oxidation of amygdalin<sup>86</sup> produces the anticipated two mols. of formic acid, thus verifying the presence of a 1 : 6-linkage in the disaccharide part of the molecule. Hexose diphosphoric acid on oxidation<sup>87</sup> yields 2 mols. of formic acid and one mol. each of phosphoglycollaldehyde and phosphoglycollic acid.<sup>88</sup> Melezitose (LXIX) has been oxidised<sup>89</sup> by sodium periodate with consumption of 4 mols. of periodate and formation of 2 mols. of formic acid and a tetra-aldehyde (LXX) which furnishes *d*-fructose on hydrolysis, thus confirming the structure of the trisaccharide.



<sup>82</sup> C. F. Heubner, R. Lohmas, R. J. Dimler, S. Moore, and K. P. Link, *J. Biol. Chem.*, 1945, **159**, 502.

<sup>82a</sup> D. B. Sprinson and E. Chargaff, *ibid.*, 1946, **164**, 433.

<sup>83</sup> G. Lindstedt, *Nature*, 1945, **156**, 448.

<sup>84</sup> (Mme.) Y. Khouvine and G. Arragon, *Bull. Soc. chim.*, 1941, **8**, 676; *Compt. rend.*, 1941, **212**, 167.

<sup>85</sup> P. Fleury and J. Courtois, *Bull. Soc. chim.*, 1943, **10**, 245; *Compt. rend.*, 1942, **214**, 366; 1943, **216**, 65.

<sup>86</sup> J. Courtois and A. Valentino, *Bull. Soc. Chim. biol.*, 1944, **26**, 469.

<sup>87</sup> J. Courtois, *Bull. Soc. chim.*, 1942, **9**, 136.

<sup>88</sup> P. Fleury and J. Courtois, *ibid.*, 1941, **8**, 69; J. Courtois and M. Ramet, *ibid.*, 1944, **11**, 539.

<sup>89</sup> N. K. Richtmeyer and C. S. Hudson, *J. Org. Chem.*, 1946, **11**, 610.

*Polyhydric Alcohols.*

(Continued from *Annual Reports*, 1943, **40**, 119.)

The reactions of polyhydric alcohols and their derivatives continue to interest many workers. 2 : 3-4 : 5-Diisopropylidene xylitol,<sup>90</sup> 2 : 4-benzylidene xylitol, 2 : 4-3 : 5-dimethylene and 2 : 4-methylene xylitol,<sup>91</sup> and 1 : 3-2 : 4-dimethylene and 2 : 4-methylene adonitol<sup>92</sup> have been isolated and their structures proved. W. T. Haskins and others<sup>93</sup> have prepared 1 : 3-2 : 5-dimethylene (LXXI) and 2 : 5-methylene *l*-rhamnitol, and have confirmed these structures by degradative and synthetical reactions. R. M. Hann and C. S. Hudson<sup>94</sup> suggest that formation of benzylidene and methylene derivatives is favoured by the presence of secondary alcohol groups in the  $\beta$ -position and *cis*- to one another, or in the  $\gamma$ -position and *trans*- to one another, and by the presence of a primary and secondary alcohol group in the  $\beta$ -position to one another. These rules are subject to uncertainties due to equilibria and differing solubilities (cf. 1 : 3-2 : 4-5 : 6-trimethylene sorbitol). In the hexitol series dimethylene *l*-iditol has been identified as the 2 : 4-3 : 5-derivative,<sup>95</sup> and the structure of triisopropylidene *d*-mannitol has been shown to be (LXXII).<sup>96</sup> This compound on graded hydrolysis yields 3 : 4-isopropylidene mannitol or 1 : 2-3 : 4-diisopropylidene mannitol (LXXIII). The latter is converted into diisopropylidene *aldehydo-d*-arabinose (LXXIV) on oxidation with lead tetra-acetate, and *d*-mannitol thus becomes a convenient source for the preparation of *d*-arabinose.

Anhydro-derivatives of many of the sugar alcohols have been prepared and their constitution proved. Dehydration of xylitol<sup>97</sup> in the presence of an acid catalyst yields anhydroxylitol, which possesses a five-membered ring since it is oxidised with consumption of one mol. of periodic acid without the formation of formaldehyde or formic acid. Sorbitol and mannitol on dehydration each yield anhydro-derivatives which possess six- or five-membered rings. Dehydration of sorbitol yields polygalitol (1 : 5-ring) (LXXV) and arlitan (1 : 4-ring) (LXXVI); the structure of the latter follows from a study of its oxidation with lead tetra-acetate which yields formaldehyde, and by the synthesis of its tetramethyl ether from the dehydration of 2 : 3 : 5 : 6-tetramethyl sorbitol.<sup>98</sup> Walden inversion does not occur during this ring formation since the tetramethyl ether of (LXXVI) has not the pro-

<sup>90</sup> R. M. Hann, A. T. Ness, and C. S. Hudson, *J. Amer. Chem. Soc.*, 1944, **66**, 73; 1946, **68**, 1769.

<sup>91</sup> R. S. Tipson and L. H. Crotcher, *J. Org. Chem.*, 1943, **8**, 95; *J. Amer. Chem. Soc.*, 1944, **66**, 670.

<sup>92</sup> R. M. Hann and C. S. Hudson, *ibid.*, p. 1906.

<sup>93</sup> W. T. Haskins, R. M. Hann, and C. S. Hudson, *ibid.*, 1945, **67**, 1800.

<sup>94</sup> R. M. Hann and C. S. Hudson, *ibid.*, p. 1909.

<sup>95</sup> *Idem*, *ibid.*, p. 602.

<sup>96</sup> L. F. Wiggins, *J.*, 1946, 13.

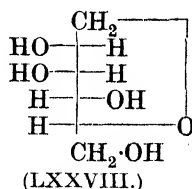
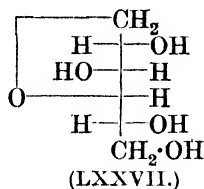
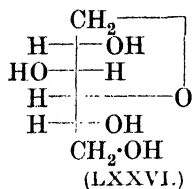
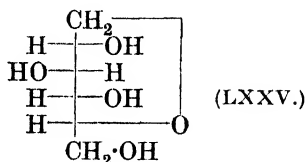
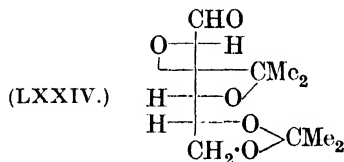
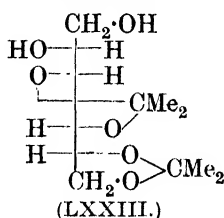
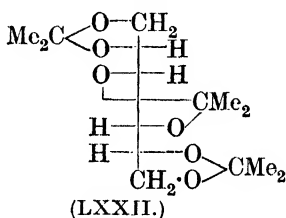
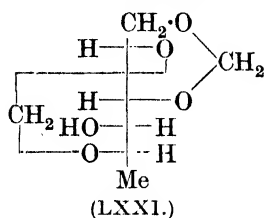
<sup>97</sup> J. F. Carson and W. D. MacLay, *J. Amer. Chem. Soc.*, 1945, **67**, 1808.

<sup>98</sup> L. N. Owen, *Ann. Reports*, 1943, **40**, 121; S. Soltzberg, R. M. Goepf, jnr., and W. Freudenberg, *J. Amer. Chem. Soc.*, 1946, **68**, 919.



perties of the corresponding dulcitol derivative (LXXVII), the synthesis of which has been described.<sup>99</sup>

Dehydration of mannitol yields a mixture of products from which styracitol (1:5-ring) (LXXVIII), 1:4-anhydromannitol (LXXIX), and 2:5-anhydrosorbitol (LXXX) were isolated. This last compound must have been produced by a Walden inversion of the hydroxyl group on C<sub>2</sub><sup>100</sup> of the mannitol molecule. *iso*Mannide (LXXXI), a 1:4-3:6-dianhydride of mannitol, is also produced during the dehydration of mannitol. The structure of this substance has been proved by L. F. Wiggins<sup>1</sup> and by R. C. Hockett and E. L. Sheffield, who also prepared an isomeric *neomannide* (a 1:5-3:6-dianhydride derivative) (LXXXII) by dehydration of styracitol<sup>2</sup> (LXXVIII). *neo*Mannide does not react with lead tetra-acetate and therefore contains no contiguous hydroxyl groups. Sorbitol similarly forms a 1:4-3:6-dianhydro-derivative, *isosorbide*<sup>3</sup> (LXXXIII, R = H). The corresponding dimethyl ether (LXXXIII, R = Me) was synthesised by R. Montgomery and L. F. Wiggins<sup>4</sup> from 2:5-dimethyl 3:6-anhydrosorbitol (LXXXIV), prepared by the reduction of 2:5-dimethyl 3:6-anhydroglucose.



The action of alkali on the 1-*p*-toluenesulphonyl 4-acetyl derivative of (LXXXIV) yielded a product (LXXXIII, R = Me) identical with that

<sup>99</sup> R. C. Hockett, M. Conley, M. Yusem, and R. I. Mason, *J. Amer. Chem. Soc.*, 1946, **68**, 922.

<sup>100</sup> R. C. Hockett, H. G. Fletcher, jr., E. L. Sheffield, R. M. Goepf, jr., and S. Soltzberg, *ibid.*, p. 930; R. C. Hockett, M. Zief, and R. M. Goepf, jr., *ibid.*, p. 935.

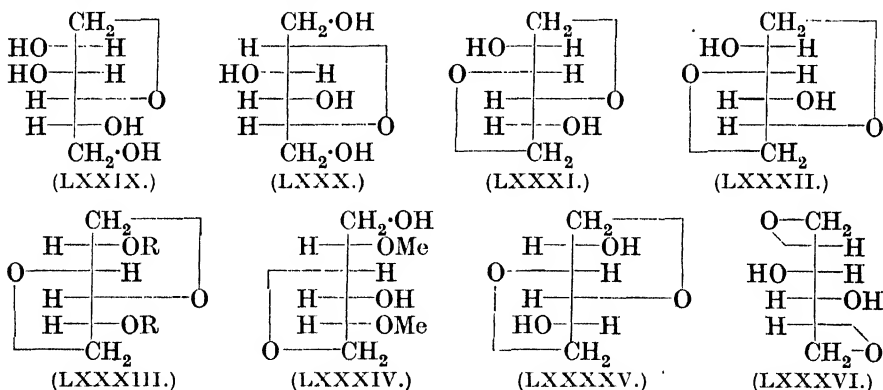
<sup>1</sup> *J.*, 1945, 4.

<sup>2</sup> *J. Amer. Chem. Soc.*, 1946, **68**, 937.

<sup>3</sup> R. C. Hockett, H. G. Fletcher, jr., E. L. Sheffield, and R. M. Goepf, jr., *ibid.*, p. 927.

<sup>4</sup> *J.*, 1946, 390.

obtained on methylation of *isosorbide*. Both *isosorbide* and *isomannide* on dehydrogenation followed by hydrogenation yield *l-isoidide* (LXXXV) by epimerisation at C<sub>5</sub> and at C<sub>2</sub> and C<sub>5</sub> respectively. *d-Iditol* on dehydration yields the optical isomer, *d-isoidide*, which has been synthesised from *d-mannitol* by the elimination of the *p*-toluenesulphonyl grouping from the 3 : 4-di-*p*-toluenesulphonate with accompanying Walden inversion of the hydroxyl groups on C<sub>3</sub> and C<sub>4</sub>. L. F. Wiggins<sup>5a</sup> describes the preparation of 5 : 6-anhydromannitol and 5 : 6-anhydrosorbitol.<sup>5b</sup> These substances are prepared by the removal with alkali of *p*-toluenesulphonic acid residues from 5-acetyl 1 : 2-3 : 4-diisopropylidene mannitol 6-*p*-toluenesulphonate and from 1 : 3-2 : 4-diethylidene sorbitol similarly substituted on C<sub>5</sub> and C<sub>6</sub>, followed by hydrolysis of the protecting acetone and acetaldehyde groupings. Derivatives of 1 : 2-5 : 6-dianhydromannitol (LXXXVI), an isomer of *isomannide*, have been prepared from 1 : 6-dichloro 3 : 4-isopropylidene and 1 : 6-diiodo 3 : 4-ethylidene mannitol. With methyl-alcoholic ammonia these substances give derivatives of 1 : 6-diamino mannitol which can be converted into resins by heating with phthalic acid.



Sugars containing ethylene oxide rings are widely used in the preparation of derivatives of the carbohydrates. J. Honeyman<sup>6</sup> has shown that *l-arabinose* can be converted into *l-xylose* by way of *isopropylidene β-methyl-l-arabinoside* and its 2-*p*-toluenesulphonate (LXXXVII), and thence by removal of the *p*-toluenesulphonyl residue with sodium methoxide into 2 : 3-anhydro-β-methyl-*l*-riboside (LXXXVIII). This is the first time that this type of reaction has been applied to the pentose sugars. In a similar manner, 2-*p*-toluenesulphonyl methyl-*d*-galactoside has been converted, by the inversion of the hydroxyl groups on C<sub>2</sub> and C<sub>3</sub>, into derivatives of *d*-idose.<sup>7, 8</sup>

Just as the configuration of the amino-group in *d*-glucosamine (LXXXIX) was related to *d*-glucose by the opening of an anhydro-ring with accompanying

<sup>5a</sup> *J.*, 1946, 384, 388.

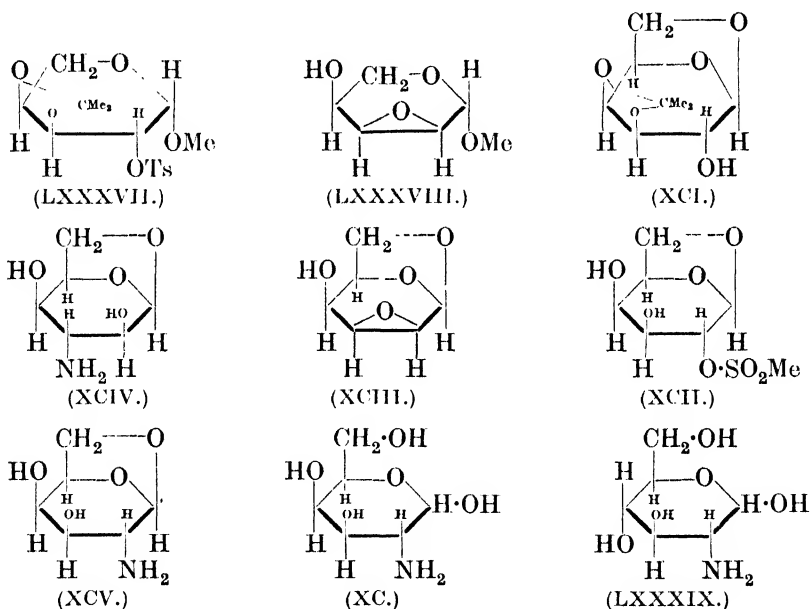
<sup>5b</sup> L. Vargha and T. Puskás, *Ber.*, 1943, **76**, 859.

<sup>6</sup> *J.*, 1946, 990.

<sup>7</sup> L. F. Wiggins, *ibid.*, 1944, 522.

<sup>8</sup> E. Sorkin and T. Reichstein, *Helv. Chim. Acta*, 1945, **28**, 1.

Walden inversion,<sup>9</sup> so the configuration of the amino-group in chondrosamine (XC) has been proved to be that of the corresponding hydroxyl group in galactose by a similar procedure. In this proof of the configuration of chondrosamine, 3 : 4-*isopropylidene* 1 : 6-anhydro-*d*-galactose<sup>10</sup> (XCI) was converted into the 2-methanesulphonyl derivative, which on mild acid hydrolysis gave 2-methanesulphonyl 1 : 6-anhydro-*d*-galactose (XCII), converted by reaction with sodium methoxide and with an accompanying Walden inversion into 2 : 3-1 : 6-dianhydro- $\beta$ -*d*-talose (XCIII). This compound with ammonia yielded, again with Walden inversion, a mixture of 3-amino 1 : 6-anhydro- $\beta$ -*d*-idose<sup>7</sup> (XCIV) and 2-amino 1 : 6-anhydro- $\beta$ -*d*-galactose (XCV). The latter substance on hydrolysis yielded chondrosamine identical with the naturally occurring product,<sup>11, 12, 13</sup> derivatives of which have been described.<sup>14</sup> *N*-Methyl-*l*-glucosamine has been isolated from the hydrolysis



products of streptomycin.<sup>15</sup> This amino-sugar is a derivative of the optical isomer of the *d*-glucosamine normally encountered. Removal of the amino-

<sup>9</sup> S. Peat, *Ann. Reports*, 1939, **36**, 270.

<sup>10</sup> D. McCreath and F. Smith, *J.*, 1939, 387.

<sup>11</sup> R. M. Hann and C. S. Hudson, *J. Amer. Chem. Soc.*, 1942, **64**, 2435.

<sup>12</sup> S. P. James, F. Smith, M. Stacey, and L. F. Wiggins, *J.*, 1946, 625; *Nature*, 1945, **156**, 308.

<sup>13</sup> P. A. Levene, "Hexosamines and Mucoproteins", Longmans Green & Co., London, 1925.

<sup>14</sup> M. Stacey, *J.*, 1944, 272.

<sup>15</sup> F. A. Kuehl, jr., E. H. Flynn, F. W. Holly, R. Mazingo, and K. Folkers, *J. Amer. Chem. Soc.*, 1946, **68**, 536; M. L. Wolfrom, A. Thompson, and I. R. Hooper, *ibid.*, p. 2343.

group from an amino-sugar by deamination with nitrous acid results in the formation of an anhydro-ring with accompanying Walden inversion.<sup>16</sup> This type of reaction is encountered when nitrous acid reacts with deacetylated chitin.<sup>17</sup> Anhydro-rings are also formed, with accompanying Walden inversion, when sugar nitrates are submitted to alkaline hydrolysis, according to E. K. Gladding and C. B. Purves.<sup>18</sup> B. Helferich and his co-workers<sup>19</sup> have shown, in a series of papers, that methanesulphonyl chloride will react with sugar derivatives with formation of well-defined crystalline products. The methanesulphonates behave like the *p*-toluenesulphonyl derivatives towards alkaline reagents and towards sodium iodide.<sup>7</sup> E. G. V. Percival and R. B. Duff<sup>20</sup> have shown that removal of a sulphuric acid residue from a C<sub>6</sub> sulphate ester of a sugar may result in the formation of a product containing an anhydro-ring. Since sugar derivatives containing sulphuric acid residues are known to occur in nature, a possible mechanism of interconversion in the sugar series becomes available.

It is of interest to note that the dilactone derived from *mannosaccharic* acid<sup>21</sup> (XCVI) has the 1 : 4-3 : 6-ring structure present in *neomannide* and *isosorbide* and in *saccharodilactone*<sup>22, 23</sup> (XCVII). Both these dilactones and the corresponding monolactones possess very interesting reactions owing to their ability to form enolic compounds (XCVIII) (XCIX; R = H) with structures possessing some of the properties of ascorbic acid (cf. ethyl hydrogen mucate)<sup>23a</sup> Glucurone<sup>24</sup> (C), the lactone of *d*-glucuronic acid, also possesses the 1 : 4-3 : 6-ring structure, since methylation yields 2 : 5-dimethyl glucurone identified after further methylation followed by oxidation as 2 : 3 : 5-trimethyl *d*-saccharic acid. Under carefully controlled conditions it may be converted in part into 2 : 5-dimethyl  $\Delta^4$ -glucosaccharo-3 : 6-lactone<sup>25</sup> (XCIX; R = Me). Tetra-acetyl glucosone hydrate (I) has been converted into kojic acid<sup>26</sup> (II). The syntheses of glucuronic, mannuronic, and galacturonic acids and of their derivatives have also been described.<sup>27, 28</sup>

It is well known that the melting points of the sugar osazones are unsatisfactory as a means of characterisation. R. M. Hann and C. S. Hudson<sup>29</sup>

<sup>16</sup> L. F. Wiggins, *Nature*, 1946, **157**, 300.

<sup>17</sup> H. Wehrli, 1937, Thesis, p. 974, Geneva.

<sup>18</sup> *J. Amer. Chem. Soc.*, 1944, **66**, 76; cf. J. Dewar, G. Fort, and N. McArthur, *J.*, 1944, 499.

<sup>19</sup> B. Helferich and H. Jochinke, *Ber.*, 1940, **73**, 1049; 1941, **74**, 719; B. Helferich and F. von Stryk, *ibid.*, 1941, **74**, 1794.

<sup>20</sup> *Nature*, 1946, **158**, 29.

<sup>21</sup> W. N. Haworth, (Miss) D. Heslop, (Miss) E. Salt, and F. Smith, *J.*, 1944, 217; (Miss) D. Heslop and F. Smith, *ibid.*, pp. 574, 577.

<sup>22</sup> F. Smith, *J.*, 1944, 510, 571, 633, 637.

<sup>23</sup> W. N. Haworth and W. G. M. Jones, *J.*, 1944, 65.

<sup>23a</sup> F. Ferraboschi, *J.*, 1909, **95**, 1248. <sup>24</sup> F. Smith, *J.*, 1944, 584.

<sup>25</sup> H. Kiliani, *Ber.*, 1943, **76**, 540. <sup>26</sup> M. Stacey and L. M. Turton, *J.*, 1946, 661.

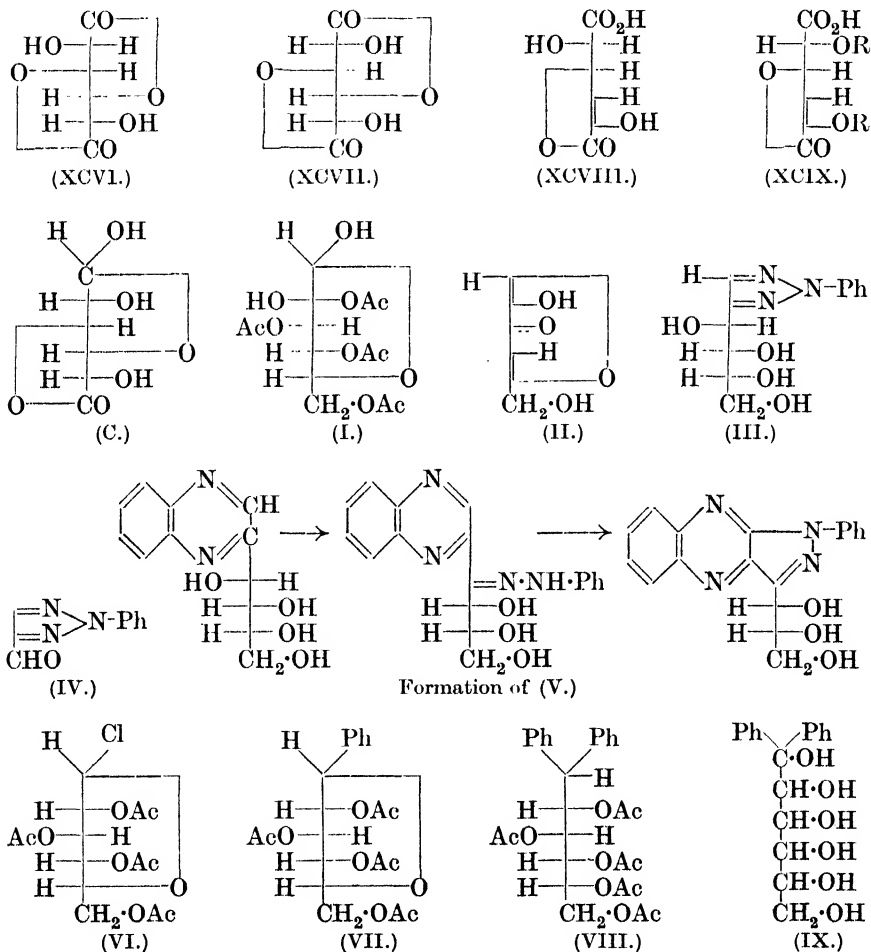
<sup>27</sup> F. Smith, M. Stacey, and P. I. Wilson, *J.*, 1945, 131; M. Stacey and P. I. Wilson *ibid.*, p. 587.

<sup>28</sup> H. S. Isbell and H. L. Frush, *J. Res. Nat. Bur. Stand.*, 1944, **32**, 77.

<sup>29</sup> *J. Amer. Chem. Soc.*, 1944, **66**, 735; 1945, **67**, 939; 1946, **68**, 1766.

have found that osazones are converted into well-characterised crystalline triazole derivatives on oxidation with copper sulphate solution. The structure of the product (III) from glucosazone was confirmed by oxidation with sodium periodate which yielded two mols. of formic acid, formaldehyde, and 4-formyl-2-phenylsotriazole (IV). G. Neümüller<sup>30</sup> has investigated the 1-phenylflavazole (V) derivatives of H. Ohle<sup>31</sup> with the object of using them to determine the constitution of the oligosaccharides. These substances are prepared by heating the sugar with a mixture of *o*-phenylenediamine and phenylhydrazine. The phenylhydrazine acts as an oxidising agent as well as forming part of the flavazole molecule.

$\alpha$ -D-Acetochloroglucose (VI) reacts with benzene in the presence of alumin-



<sup>30</sup> *Arkiv Kemi, Min. Geol.*, 1946, **21A**, No. 19, 13.

<sup>31</sup> H. Ohle and G. A. Milkonian, *Ber.*, 1941, **74**, 279, 398.

ium chloride<sup>32</sup> to yield first tetra-acetyl *d*-glucopyranosylbenzene (VII) and then the penta-acetyl derivative of 1:1-diphenyl 1-deoxysorbitol (VIII) which may also be obtained by the action of phenylmagnesium bromide<sup>33</sup> on acetochloroglucose; on oxidation it yields benzophenone. The related 1:1-diphenyl hexitols (IX) derived from *d*-glucose and *d*-galactose had been prepared by C. Paal and F. Hornstein and by C. Paal and E. Weidenkaff<sup>34</sup> from phenylmagnesium bromide and tetra-acetyl *d*-glucono- or *d*-galactono-lactones. It is possible that the reaction between glucose or cellulose and benzene in the presence of concentrated sulphuric acid leads to a similar type of product.<sup>35</sup>

#### *Disaccharides and Polysaccharides.*

The synthesis of sucrose by purely chemical means has so far evaded the chemist. A notable advance, however, has been made by W. Z. Hassid, M. Doudoroff, and H. A. Baker,<sup>36</sup> who have prepared it enzymatically by condensation of the potassium salt of *d*-glucose 1-phosphate and *d*-fructose with the aid of the phosphorylase of the bacterium *Pseudomonas saccharophila*. Replacement of *d*-fructose by *l*-sorbose<sup>37</sup> results in the synthesis of a new non-reducing disaccharide (X),  $\alpha$ -*d*-glucopyranosido- $\alpha$ -*l*-sorbofuranoside which shows many of the reactions of sucrose. Its constitution was proved by the observations that it reacted with three mols. of periodic acid with the formation of one mol. of formic acid, and that it was rapidly hydrolysed by dilute acid, indicating a furanose link between the sugars. This synthesis indicates that the *d*-glucose linkage is of the  $\alpha$ -type. *d*-Xyloketose<sup>38</sup> similarly condenses with the potassium salt of *d*-glucose 1-phosphate with the formation of a new type of disaccharide (XI) analogous to sucrose, in which the *d*-fructofuranose part of the molecule is replaced by *d*-ketoxylofuranose. The work of A. Gottschalk is of interest in this connection; he has found that at 20° an equilibrated solution of fructose in 0.1M-phosphate solution contains about one-fifth of the furanose form which is fermentable; the remaining fructopyranose is not fermentable. The synthesis by purely chemical means of  $\beta\beta$ -trehalose,  $\beta$ -vicianose, and  $\beta$ -primeverose has also been reported.<sup>39</sup>

It has been found that sucrose and maltose behave like the monosaccharides in that they react with paraldehyde in the presence of an acid catalyst with the formation of ethylidene derivatives.<sup>40</sup>

The oxidative degradation of polysaccharides has been used to obtain

<sup>32</sup> C. D. Hurd and W. A. Bonner, *J. Amer. Chem. Soc.*, 1945, **67**, 1664, 1759.

<sup>33</sup> *Idem*, *ibid.*, p. 1972.

<sup>34</sup> *Ber.*, 1906, **39**, 2823, 2827.

<sup>35</sup> A. M. Natsakoff, *J. Russ. Phys. Chem. Soc.*, 1902, **34**, 231; 1912, **44**, 1152; *Ann. Reports*, 1912, **9**, 99.

<sup>36</sup> *J. Amer. Chem. Soc.*, 1944, **66**, 1416.

<sup>37</sup> *Ibid.*, 1945, **67**, 1394.

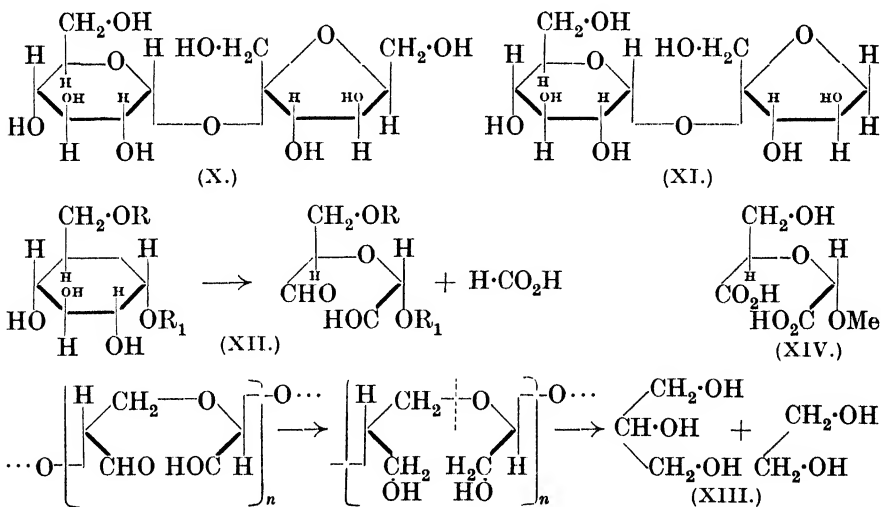
<sup>38</sup> *Ibid.*, 1946, **68**, 1465.

<sup>39</sup> *Australian J. Expt. Biol. Med. Sci.*, 1943, **21**, 133.

<sup>40</sup> G. H. Coleman and C. H. McCloskey, *J. Amer. Chem. Soc.*, 1943, **65**, 1778.

<sup>40</sup> R. Sutra, *Bull. Soc. chim.*, 1942, **9**, 794; cf. K. Hess and W. Granberg, *Ber.*, 1939, **72**, 1896, 1906.

insight into the structure of the polysaccharides.<sup>41</sup> Inspection reveals that any sugar residue which contains two or more hydroxyl groups on adjacent carbon atoms will react with the periodate ion. If free hydroxyl groups are present on each of three adjacent carbon atoms, two mols. of periodate will be consumed and one mol. of formic acid will be produced per mol. of sugar. An estimate of the amount of formic acid produced will thus give a measure of the number of pyranose sugar residues (XII) linked through C<sub>1</sub> or C<sub>1</sub> and C<sub>6</sub> only. This method has been used in the determination of the percentage of end groups in glycogen, starch, and amylopectin. The results show that these polysaccharides contain, at the most, traces only of glucose residues which are linked solely through carbon atoms 1 and 6, since the amount of formic acid produced is in close agreement with the figure calculated from the amounts of end group isolated after methanolysis of the methylated polysaccharide. The method can be applied to polysaccharides which contain linkages other than the 1 : 4-linkage present in starch and glycogen. V. C. Barry<sup>42a</sup> and T. Dillon<sup>42b</sup> have shown that in a polysaccharide built up entirely of 1 : 3-linked pyranose residues, such as laminarin, oxidation does not occur, as the requisite  $\alpha$ -glycol system is not present except at the reducing and non-reducing end of the molecule.



K. Ahlborg<sup>43</sup> has used lead tetra-acetate and periodic acid in attempts to distinguish between 1 : 4-, 1 : 6-, and 1 : 1-linked disaccharides. The method was used also to determine the type of linkages and their relative positions in dextrans by studying the rate and type of oxidation.

The oxidation of xylan with periodic acid and its salts under a variety of

<sup>41</sup> F. Brown, S. Dunstan, T. G. Halsall, E. L. Hirst, and J. K. N. Jones, *Nature*, 1945, **156**, 785.

<sup>42a</sup> V. C. Barry, *ibid.*, 1943, **152**, 538.

<sup>42b</sup> T. Dillon, *ibid.*, 1945, **155**, 546.

<sup>43</sup> *Svensk Kem. Tidskr.*, 1942, **54**, 205.

conditions has been discussed by G. Jayme and M. Satre <sup>44</sup> in a series of publications. These workers found that xylan, on graded hydrolysis, yielded 2.7% of *l*-arabinose and that on periodate oxidation, followed by hydrolysis, nearly 70% of the theoretical yield of glyceraldehyde was produced. This figure was raised to 85% on oxidation with a buffered periodate solution. Hydrogenation of the oxidised xylan gave, after hydrolysis, a mixture of glycerol and ethylene glycol (XIII). Small amounts of xylose which are produced on hydrolysis of oxidised xylan may arise, not from incomplete oxidation of the polysaccharide as suggested by G. Jayme, but from the xylose residues which are involved in branched chain formation and are substituted on C<sub>1</sub>, C<sub>3</sub>, and C<sub>4</sub>.<sup>45</sup> These sugar residues do not contain free hydroxyl groups on adjacent carbon atoms and would therefore be unaffected by the periodate reagent.

The oxidation of cellulose with buffered periodate solution has been studied by G. Jayme and S. Maris <sup>46</sup> and found to follow a course very similar to that observed for xylan. In agreement with the results of G. F. Davidson <sup>47</sup> it was found that no definite halt occurred in the oxidation. The type of oxidation encountered and the physical changes observed in the cellulose fibres varied with the pH of the solution.<sup>48</sup> Hydrolysis of the resultant polymeric aldehyde yielded glyoxal and *d*-erythrose, whilst erythritol was produced on hydrolysis of the hydrogenated polymeric aldehyde.

The atmospheric oxidation of  $\alpha$ -methyl-*d*-glucoside <sup>49</sup> in ammoniacal cupric solution has been studied; the product (XIV) isolated demonstrates that the glucose residue undergoes, under these conditions, an oxidation similar to that encountered with periodic acid. It is suggested that this type of oxidation is not responsible for loss of viscosity of cellulose solutions <sup>50</sup> in the Schweitzer reagent, since glucuronic acid and not (XIV) was the main product of oxidation encountered; in support of this theory it was found that 6-trityl cellulose <sup>51</sup> in cupra-ammonium solution was not oxidised under these conditions.

Sodium in liquid ammonia degrades methylated cellulose, with loss of methoxyl groupings, according to N. N. Schorigina,<sup>52</sup> who states that methylation in liquid ammonia with sodium and methyl iodide, according to the method of I. E. Muskat,<sup>53</sup> is unsatisfactory, and that K. Freudenberg's

<sup>44</sup> *Ber.*, 1942, **75**, 1840; 1944, **77**, 242, 248; *Papier-Fabr. Wochb. Papierfabrik*, 1944, 295.

<sup>45</sup> R. A. S. Bywater, W. N. Haworth, E. L. Hirst, and S. Peat, *J.*, 1937, 1983.

<sup>46</sup> *Ber.*, 1944, **77**, 382.

<sup>47</sup> *J. Textile Inst.*, 1941, **32**, T, 109.

<sup>48</sup> G. Goldfinger, H. Mark, and S. Siggia, *Ind. Eng. Chem.*, 1943, **35**, 1083.

<sup>49</sup> V. I. Ivanov and K. M. Sokova, *Compt. rend. Acad. Sci. U.R.S.S.*, 1944, **42**, 175.

<sup>50</sup> V. I. Ivanov and E. D. Kaverzneva, *ibid.*, 1945, **48**, 405.

<sup>51</sup> E. D. Starkheeva-Kaverzneva and V. I. Ivanov, *Bull. Acad. Sci. U.R.S.S.*, 1945 **603**; *Uspekhi Khim.*, 1944, **13**, 281.

<sup>52</sup> *J. Gen. Chem. Russia*, 1944, **14**, 825.

<sup>53</sup> *J. Amer. Chem. Soc.*, 1934, **56**, 693.



method<sup>54</sup> for the determination of molecular size by end-group assay may thus give low yields of tetramethyl methylglucoside.

Oxidation of cellulose with dinitrogen tetroxide converts the terminal alcohol groups into carboxyl groups with the resultant production of a poly-uronic, which has useful medical applications. The formation of aldehyde- and carboxyl groups in the oxidised polysaccharide has been followed by studying the condensation of the oxidised polymer with *p*-nitroaniline or hydrazine derivatives, and from the yields of carbon dioxide produced from the uronic acid residues on boiling with acid.<sup>55, 56</sup>

Amorphous cellulose has been converted, by treatment with water, into crystalline cellulose,<sup>57</sup> the degree of conversion being investigated by X-ray diffraction patterns, and by comparing the integral heats of wetting and sorption isotherms of the original fibres, and dry amorphous and recrystallised powders. An evaluation of the percentage of amorphous cellulose in a mixture of crystalline and amorphous material can be made from a determination of the number of hydroxyl groups in the polysaccharide, which react with thallos ethoxide dissolved in benzene.<sup>58</sup> The figure obtained is not affected by the presence of *n*-hydrocarbons but varies as the molecular weight of any solvent ether added. An estimate of the amount of crystalline material present in a cellulose sample can also be made from a study of the velocity of nitration of the polysaccharide<sup>59</sup> with nitric acid-sulphuric acid-water mixtures of various compositions, the amorphous material being the more easily nitrated.

Bacterial cellulose<sup>60</sup> from *B. Xylinium* has been examined; it appears to consist of crystalline fibrilles of varying size and degree of orientation. It would appear that the majority of the reactions of cellulose and its derivatives are micellar,<sup>61</sup> not macromolecular, in character. When, by special methods, the micelles are dispersed as macromolecules, all the functional groups are reactive, whereas normally those in the interior of the micelles do not react since they are inaccessible.

The effect of salts and solvents on the acetyl derivatives of cellulose has been investigated.<sup>62</sup> Calcium chloride is observed to increase the viscosity of cellulose acetate in methyl ethyl ketone but to be without effect in *m*-cresol. Acetone forms a molecular compound with cellulose acetate consisting of ten parts of cellulose acetate and three parts of acetone.<sup>63</sup> It has been found

<sup>54</sup> K. Freudenberg and H. Boppel, *Ber.*, 1938, **71**, 2505; K. Hess, H. Schulze, and B. Kranjc, *Ber.*, 1940, **73**, 1069.

<sup>55</sup> K. Maurer and G. Reiff, *J. Makromol. Chem.*, 1943, **1**, 27; K. Maurer, *Helv. Chim. Acta*, 1946, **29**, 130.

<sup>56</sup> F. Geiger and A. Wissler, *ibid.*, 1945, **28**, 1638.

<sup>57</sup> P. H. Hermans and A. Weidinger, *J. Amer. Chem. Soc.*, 1946, **68**, 1138.

<sup>58</sup> A. G. Assaf, R. H. Hass, and C. B. Purves, *ibid.*, 1944, **66**, 59.

<sup>59</sup> J. Chédin, *Memorial des Services Chimiques de l'État (Paris)*, 1944, 154; *Chim. et Ind.*, 1946, July 7th.

<sup>60</sup> E. Franz and E. Schiebold, *J. Makromol. Chem.*, 1943, **1**, 4.

<sup>61</sup> T. Lieser, *Ber.*, 1941, **74**, 708. <sup>62</sup> H. Lohman, *J. pr. Chem.*, 1940, **155**, 299.

<sup>63</sup> L. Clément and C. Rivière, *Bull. Soc. chim.*, 1943, **10**, 386.

that partly methylated cellulose will react with oxalyl chloride in chloroform containing dimethyl-*p*-toluidine<sup>64</sup> with the formation of cross-linkages. The formation of gels is dependent upon the concentration of the reagents.

Glycogens from yeast,<sup>65</sup> mussel muscle,<sup>66</sup> *Ascaris lumbricoides*, human muscle, and rabbit liver<sup>41</sup> have been investigated and the ratio of terminal to non-terminal glucose residues found to be *ca.* 1 to 12, except in rabbit liver glycogen, where the figure may approach 1 to 18. These results have been obtained by end-group assay, both by the methylation method and from the yield of formic acid produced on oxidation of the polysaccharide with the periodate ion. Oxidation of glycogen<sup>67</sup> with alkaline potassium hypobromite solution results in the formation of maltosidogluconic acid and maltobionic acid. These acids are also produced when starch is oxidised by the same reagent.

Several methods are now available for separating amylose from starch. These are the original techniques of M. Samec,<sup>68</sup> M. E. Baldwin,<sup>69</sup> and T. J. Schoch,<sup>70</sup> and improved methods in which thymol,<sup>71</sup> chloral, or nitroparaffins<sup>72</sup> are used. The reported separation of amylose from starch by adsorption on cotton wool<sup>73</sup> has been confirmed.<sup>73a</sup> For separation to be effected, the formation of a complex with a substance possessing an H-donor or H-acceptor group is necessary.<sup>74</sup> This successful isolation of pure amylose from starch has been facilitated by the development of an elegant potentiometric titration method<sup>75</sup> which may be used to determine the purity of the isolated amylose. It is found that the fatty acids<sup>76</sup> will form complexes with amylose and that exhaustive extraction with a water-dioxan mixture is necessary in order to remove completely this material which otherwise interferes with the potentiometric iodine titration of amylose. The amylose-iodine complex has been examined spectroscopically, and by this means it has been found possible to differentiate amylose from amylopectin. This difference in

<sup>64</sup> R. Signer and P. von Taval, *Helv. Chim. Acta*, 1943, **26**, 1972.

<sup>65</sup> R. Jeanloz, *ibid.*, 1944, **27**, 1501.

<sup>66</sup> K. H. Meyer and J. Prins, *ibid.*, 1941, **24**, 58; K. H. Meyer and R. Jeanloz, *ibid.*, 1943, **26**, 1784.

<sup>67</sup> C. Dumazert and R. Senequier, *Bull. Soc. Chim. biol.*, 1945, **27**, 446.

<sup>68</sup> *Kolloid-Beih.*, 1921, **13**, 272.

<sup>69</sup> *J. Amer. Chem. Soc.*, 1930, **52**, 2907; K. Meyer, W. Bretans, and P. Bernfield, *Helv. Chim. Acta*, 1940, **23**, 845.

<sup>70</sup> *J. Amer. Chem. Soc.*, 1942, **64**, 2957.

<sup>71</sup> W. N. Haworth, S. Peat, and P. E. Sagrott, *Nature*, 1946, **157**, 19.

<sup>72</sup> R. L. Whistler and G. E. Hilbert, *J. Amer. Chem. Soc.*, 1945, **67**, 1161.

<sup>73</sup> C. Tanret, *Compt. Rend.*, 1914, **158**, 1353; E. Pacsu and J. W. Müller, *J. Amer. Chem. Soc.*, 1941, **63**, 1168.

<sup>73a</sup> W. R. Ashford, T. H. Evans, and H. Hibbert, *Canadian J. Res.*, 1946, **24**, B, 246.

<sup>74</sup> R. S. Bear, *ibid.*, 1944, **66**, 2122; E. Bois and G. Valtieres, *Canadian J. Res.*, 1945, **23**, 214.

<sup>75</sup> L. F. Bates, D. French, and R. E. Rundle, *J. Amer. Chem. Soc.*, 1943, **65**, 142; cf. M. Padoa and B. Savare, *Gazzetta*, 1906, **36**, 313.

<sup>76</sup> L. Lehrman, *J. Amer. Chem. Soc.*, 1945, **67**, 1541; T. J. Schoch and C. B. Williams, *ibid.*, 1944, **66**, 1232; F. F. Mikus, R. M. Hixon, and R. E. Rundle, *ibid.*, 1946, **68**, 1115; F. L. Bates, *Iowa State Coll. J. Sci.*, 1944, **19**, 6.

colour-absorption cannot be used to analyse whole starches,<sup>77</sup> unless the blue values of the pure amylose and amylopectin fractions of each individual starch are known. In this connection, it may be noted that the starch from the pea, wrinkle seeded variety, "Alderman", may contain as much as 70% of amylose<sup>78</sup> (the usual content of amylose in pea starch is *ca.* 20%). R. W. Kerr has presented evidence to show that amylose and amylopectin are heterogeneous.<sup>79</sup> From an examination of the viscosity data of fractionated amylose, he suggests that this part of some starches (particularly tapioca) may contain a small number of branch chains. K. Hess and E. Steurer<sup>80</sup> also consider that amylose is a branched-chain polysaccharide. K. Meyer,<sup>69</sup> however, is of the opinion that the amylose molecule is unbranched. The difference in properties between amylopectins from various sources, it is suggested, is not explained by a simple variation in molecular size, but by a variation in the degree of branching.

A detailed study of the enzymic hydrolysis of starch has been undertaken by (Sir) W. N. Haworth, S. Peat, and H. Kitchen.<sup>81</sup> The hydrolysis of amylose by  $\beta$ -amylase converts it completely into maltose, while amylopectin is converted into a mixture of maltose (*ca.* 46%) and  $\alpha$ -amylodextrin (limit dextrin).<sup>81</sup> This dextrin, which has an apparent unit chain length of 11–12 glucose residues, is not susceptible to further attack by  $\beta$ -amylase until it has been "sensitised" by contact with salivary amylase. The action of  $\beta$ -amylase then continues until maltose and a dextrin of apparent unit chain length of 7–8 glucose residues is produced. Two further treatments using this double procedure yield dextrins of apparent unit chain length of 5–6 and 4–5 glucose residues respectively. The mechanism of this amylolysis may be explained on the basis of the laminated formula for starch.<sup>82</sup> It is now suggested<sup>81</sup> that sensitisation with salivary amylase causes the rupture of all polymeric links in the starch-dextrin molecule, with the formation of material liable to further attack by  $\beta$ -amylase. K. H. Meyer<sup>83</sup> considers starch to be a mixture of linear amylose molecules and of amylopectin of a highly ramified structure which is degraded by  $\beta$ -amylase until a linkage other than 1 : 4- prevents further hydrolysis. Yeast maltase ( $\alpha$ -glucosidase) may then remove this inhibiting glucose residue, after which  $\beta$ -amylase continues the hydrolysis of the exposed 1 : 4-linked glucose residues until a glucose residue, linked other than through carbon atoms 1 and 4, prevents further hydrolysis. K. Myrbäck<sup>84</sup> holds similar views; he considers that the enzyme degrades all the straight-chain parts of the amylopectin molecule

<sup>77</sup> R. R. Baldwin, *Iowa State Coll. J. Sci.*, 1943, **18**, 10; R. R. Baldwin, R. S. Bear, and R. E. Rundle, *J. Amer. Chem. Soc.*, 1944, **66**, 111.

<sup>78</sup> G. E. Hilbert and M. M. McMasters, *J. Biol. Chem.*, 1946, **162**, 229.

<sup>79</sup> *J. Amer. Chem. Soc.*, 1945, **67**, 2268; 1943, **65**, 193; *Arch. Biochem.*, 1945, **7**, 392.

<sup>80</sup> K. Hess and E. Steurer, *Ber.*, 1940, **73**, 1076. <sup>81</sup> *J.*, 1943, 619.

<sup>82</sup> W. N. Haworth, E. L. Hirst, and F. A. Isherwood, *J.*, 1937, 577.

<sup>83</sup> *Helv. Chim. Acta*, 1940, **23**, 1465; *Compt. rend. Soc. phys. hist. nat. Genève*, 1940, **57**, 19; *Naturwiss.*, 1940, **28**, 564.

<sup>84</sup> K. Myrbäck, *J. pr. Chem.*, 1943, **162**, 29; K. Myrbäck and E. Leisener, *Arkiv Kemi, Min. Geol.*, 1944, **17A**, No. 18, p. 22.

until they meet an *isomaltose* residue, a branch chain, or a phosphoric acid substituent. The residual limit dextrins contain these abnormal linkages and all the phosphorus originally present in the starch. The formation of dextrins containing six units or rings of six or seven <sup>85</sup> glucose residues is explained on the structure of amylopectin in which a glucose residue is spatially near to the residue five or six units further along the chain. K. Myrbäck <sup>86</sup> have isolated *isomaltose* (an isomer of gentiobiose) from maize starch by acid hydrolysis and from a limit dextrin by enzymatic hydrolysis with taka-diaxase. They conclude that potato starch contains one *isomaltose* residue for every 15—20 maltose residues, and that amylose contains one *isomaltose* residue for every 10 maltose residues. The work of F. Brown *et al.*<sup>41</sup> is not in agreement with these conclusions. It is known that glucose on heating with acid may give a disaccharide of the gentiobiose type <sup>86a</sup> type and that evidence from enzymic degradations is liable to uncertainties owing to the possible synthetic as well as degradative actions of enzymes.<sup>86b</sup> The pyrodextrins from maize starch <sup>87</sup> appear to be different from the dextrins obtained by the action of  $\beta$ -amylase, since no Schardinger dextrins are produced by the action of *B. Macerans*. The complete enzymic hydrolysis of waxy maize starch by the amylase <sup>88</sup> from *Aspergillus Oryzae* yields some *lævo*-glucosan, but whether this is an artefact or part of the starch molecule is unknown.

The preparation of nitro-starch <sup>89</sup> has been achieved without degradation of the molecule, by carrying out the nitration under carefully controlled anhydrous conditions. Nitrogen pentoxide is the nitrating agent, and nitric acid, which is also produced in the reaction, is removed as a hydrogen-bonded complex of sodium fluoride.

The synthesis of a polysaccharide from *d*-glucose 1-phosphate and potato phosphorylase requires the presence of a trace of activator.<sup>90</sup> An examination of the relative activity of various polysaccharides and dextrins has shown that amylopectin from corn starch has the greatest activity,<sup>91</sup> and it appears that not more than six or seven glucose residues in the dextrin are necessary for activity to be observed. The cyclic Schardinger dextrins inhibit polysaccharide formation, but on partial hydrolysis with dilute acids they become activators of potato phosphorylase. When, however, the phosphorylase systems of the heart or liver are used, glycogen-like materials and not amylose may result. In this case no catalyst is required, and the formation of poly-

<sup>85</sup> D. French and R. E. Rundle, *J. Amer. Chem. Soc.*, 1943, **65**, 1707.

<sup>86</sup> *Biochem. Z.*, 1940, **307**, 49, 53, 69; 1941, **308**, 187.

<sup>86a</sup> K. Myrbäck, *Svensk Kem. Tidskr.*, 1941, **53**, 67, 269.

<sup>86b</sup> Compare the synthesising action of amylase on maltose, W. W. Pigman, *J. Res. Nat. Bur. Stand.*, 1944, **33**, 111.

<sup>87</sup> B. Brimhall, *Ind. Eng. Chem.*, 1944, **36**, 72.

<sup>88</sup> E. M. M. Montgomery and G. E. Hilbert, *J. Amer. Chem. Soc.*, 1946, **68**, 916.

<sup>89</sup> G. V. Caesar and M. Goldfrank, *ibid.*, p. 372.

<sup>90</sup> W. Z. Hassid and R. M. McCready, *ibid.*, 1941, **63**, 2171.

<sup>91</sup> Elsa C. Proehl and H. G. Day, *J. Biol. Chem.*, 1946, **163**, 667.

saccharide is autocatalytic. W. O. Kermack<sup>92</sup> likens this formation of glycogen to that of bacterial growth. In some respects it is analogous to the growth of a virus in a living organism.

An advance of the greatest significance in the chemistry of starch has been made with the synthesis of amylopectin. E. J. Bourne and S. Peat<sup>93</sup> have isolated from potato juice an enzyme (Q-enzyme) which, acting in conjunction with purified potato phosphorylase, effects the conversion of *d*-glucose 1-phosphate into amylopectin. The Q-enzyme may hydrolyse amylose with the formation of pseudo-amylose, an unbranched polymer containing 20 glucose residues linked through carbon atoms 1 and 4, which then undergoes lateral combination with the formation of the branched polymer,<sup>81</sup> amylopectin. This material possesses one end group for each 20 glucose residues, a value identical with that obtained for natural amylopectin.<sup>94</sup>

Chondroitin, a sulphate ester, which occurs in the bovine nasal septa and bovine and human trachea, contains *d*-glucuronic acid and *N*-acetyl-*d*-galactosamine, and is a branched chain polymer with the sulphate residue attached to the galactosamine moiety.<sup>95</sup> Heparin,<sup>96</sup> which is also a sulphate ester, contains *d*-glucosamine and a *d*-glucuronic acid; the amino-group of the glucosamine is not free but not acetylated (cf. *N*-methyl-*l*-glucosamine in streptomycin).

Polysaccharide sulphuric esters commonly occur in seaweeds, and V. C. Barry and T. Dillon<sup>97</sup> describe a galactan sulphuric ester from *Dilsea edulis*. One sulphuric acid residue is present for every 4—5 *d*-galactose residues. An estimation of the galactan content before and after oxidation with periodic acid shows that one galactose residue in five present in the polysaccharide is oxidised by periodic acid. This would indicate either that one galactose residue in five is an end group or that four galactose residues are linked through carbon atoms 1 and 3 and the fifth is linked through carbon atoms 1 and 2, 1 and 4, or 1 and 6. *Gigartina stellata* also contains a galactan sulphate<sup>98</sup> the properties of which are similar to those of the polysaccharide from *Dilsea edulis*. The methylated polysaccharide on hydrolysis gave 2 : 6-dimethyl *d*-galactose, while the difficulty encountered in removing the sulphuric acid grouping indicates that it is located at C<sub>4</sub>. The polysaccharide does not react with periodic acid and contains some sugar other than *d*-galactose. This may be 2-keto-*d*-gluconic acid, which has been isolated by E. G. Young and F. A. H. Rice<sup>99</sup> from the polysaccharide, carrageenin, found in Iceland

<sup>92</sup> *Science Progress*, 1946, **34**, 784.

<sup>93</sup> *J.*, 1945, 877, 882.

<sup>94</sup> K. H. Meyer, *Helv. Chim. Acta*, 1940, **23**, 865, 875; W. Z. Hassid and R. M. McCready, *J. Amer. Chem. Soc.*, 1943, **65**, 1157.

<sup>95</sup> H. G. Bray and M. Stacey, *Biochem. J.*, 1944, **38**, 142; A. Pirie, *ibid.*, 1946, **40**, Proc. XLXII.

<sup>96</sup> M. L. Wolfrom, D. I. Weisblatt, J. V. Karabinos, W. H. McNally, and J. McClean, *J. Amer. Chem. Soc.*, 1943, **65**, 2077; M. L. Wolfrom and J. V. Karabinos, *ibid.*, 1945, **67**, 679; M. L. Wolfrom and F. A. H. Rice, *ibid.*, 1946, **68**, 532.

<sup>97</sup> *Proc. Royal Irish Acad.*, 1945, **50 B**, 349.

<sup>98</sup> E. T. Dewar and E. G. V. Percival, *Nature*, 1945, **156**, 633.

<sup>99</sup> *J. Biol. Chem.*, 1944, **156**, 781; 1946, **164**, 35.

moss. *d*-Galactose is also a component of this polysaccharide, which is a sulphuric ester. The identification of 2-keto-*d*-gluconic acid as a component of the polysaccharide is of considerable biochemical interest in view of its ready conversion, by loss of carbon dioxide, into *d*-arabinose, and its relationship to isovitamin C.

The arabogalactan of the larch has been submitted to a detailed investigation by E. V. White,<sup>100</sup> who concludes that the polymer is a homogeneous substance containing *l*-arabinose and *d*-galactose in the molecular proportions of 1 to 6. Complete hydrolysis of the fully methylated polysaccharide gave 2 : 3 : 4 : 6-tetramethyl *d*-galactose, 2 : 3 : 5-trimethyl *l*-arabinose, 2 : 3 : 4-trimethyl *d*-galactose, and 2 : 4-dimethyl *d*-galactose in the proportions 2 : 1 : 1 : 3. Partial hydrolysis gave, in small yield, two disaccharides identified as the hexamethyl galactosidogalactose (XV; R = H) and the corresponding heptamethyl derivative (XV; R = Me). Graded hydrolysis of the arabogalactan or of its methyl ether caused preferential elimination of *l*-arabinose residues. Thus treated, the polysaccharide on methylation and hydrolysis then furnished a mixture of sugars in which the percentages of trimethyl *l*-arabinose and 2 : 4-dimethyl *d*-galactose had fallen, whilst a new sugar, 2 : 4 : 6-trimethyl *d*-galactose was isolated, thus proving that the arabinose residue was attached through position 6 of a galactose residue which was also linked through C<sub>1</sub> and C<sub>3</sub>. With this information available, E. V. White suggested that the polysaccharide consisted of a main chain of *d*-galactose residues linked through the hydroxyl groups on carbon atoms 1 and 6 and that each sugar unit is substituted on C<sub>3</sub> by secondary chains consisting of 1 : 3-linked galactose or galactoarabinose residues. One formula which is in agreement with the available evidence is given in (XVI).

Further evidence in support of this formula was obtained from an examination of the reaction of *p*-toluenesulphonyl chloride and triphenylmethyl chloride with the polysaccharide.<sup>1</sup> Each of these reagents forms derivatives which indicate that arabogalactan contains three CH<sub>2</sub>·OH groups per 1 arabinose and 6 galactose residues. W. G. Campbell, E. L. Hirst, and J. K. N. Jones<sup>2</sup> had previously arrived at a similar type of structure (XVII) for the galactan portion of the molecule. Evidence was given, which showed that the polysaccharide was a mixture of a galactan and araban or of a galacto-araban and galactan rather than a homogeneous polysaccharide, since fractions of varying arabinose content could be isolated. This result is in agreement with the work of L. E. Wise and his co-workers<sup>3</sup> who separated the  $\epsilon$ -galactan, by fractionation, into products of varying physical and chemical properties. The use of the ultracentrifuge has also demonstrated that this material is a mixture of two polysaccharides of different molecular weights, approximately 16,000 and 100,000.<sup>4</sup>

<sup>100</sup> *J. Amer. Chem. Soc.*, 1941, **63**, 2871; 1942, **64**, 302, 1509, 2838.

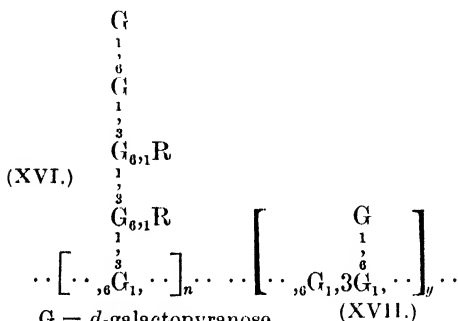
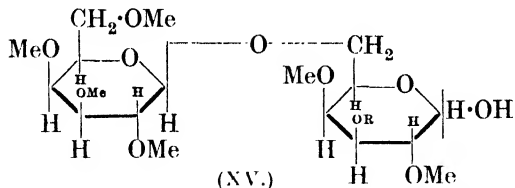
<sup>1</sup> W. Low and E. V. White, *ibid.*, 1943, **65**, 2430.

<sup>2</sup> *Nature*, 1941, **147**, 25.

<sup>3</sup> F. C. Peterson, A. J. Barry, H. Unkauf, and L. E. Wise, *J. Amer. Chem. Soc.*, 1940, **62**, 2361.

<sup>4</sup> H. Mosimann and T. Svedberg, *Kolloid-Z.*, 1942, **100**, 99.

Gum tragacanth <sup>5</sup> has been shown to be a mixture of an acidic polysaccharide, a neutral polysaccharide, and a sterol glucoside. The acidic polysaccharide is built up of *l*-fucose, *d*-xylose, and *d*-galacturonic acid residues, all of which appear to be in the pyranose form. The polysaccharide is of the branched-chain type with xylose and fucose end groups, and resembles the plant mucilages rather than the plant gums in that its acidity is due to

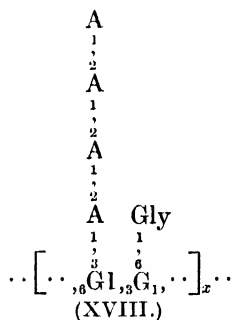


G = *d*-galactopyranose

A = *l*-arabofuranose

Gly = *d*-glucuronopyranose: united through hydroxyl groups on the numbered carbon atoms

R = G or A



*d*-galacturonic and not *d*-glucuronic acid. Hydrolysis of the methylated material yielded a mixture of sugars amongst which 2:3:4-trimethyl *l*-fucose, 2:3:4-trimethyl *d*-xylose, 3:4-dimethyl *d*-xylose, 2:3-dimethyl *d*-galacturonic acid, and a monomethyl galacturonic acid were identified. The neutral polysaccharide is an arabogalactan <sup>6</sup> which on methylation followed by hydrolysis yields 2:3:5-trimethyl *l*-arabinose, 2:3-dimethyl *l*-arabinose, *l*-arabinose, and a dimethyl *d*-galactose. In this respect it differs from the branched-chain araban <sup>7</sup> associated with pectic acid, which is built up solely of *l*-arabofuranose residues.

Mesquite gum <sup>8</sup> and damson gum <sup>9</sup> on hydrolysis each give a mixture of sugars amongst which *l*-arabinose, *d*-galactose, and *d*-glucuronic acid can be detected. Hydrolysis of methylated mesquite gum yields 2:3:5-trimethyl *l*-arabinose, 3:5-dimethyl *l*-arabinose, 2:4-dimethyl *d*-galactose, and 2:3:4-trimethyl *d*-glucuronic acid in the proportions 1:3:2:1. The gum is there-

<sup>5</sup> S. P. James and F. Smith, *J.*, 1945, 739.

<sup>6</sup> *Idem*, *ibid.*, p. 749.

<sup>7</sup> E. L. Hirst, *J.*, 1942, 71.

<sup>8</sup> E. V. White, *J. Amer. Chem. Soc.*, 1946, 68, 272.

<sup>9</sup> E. L. Hirst and J. K. N. Jones, *J.*, 1938, 1174; 1939, 1482; 1946, 506.

fore of the branched-chain type and is of relatively simple structure; one possible structure is indicated in (XVIII).

The gum from the damson tree contains *d*-mannose and *d*-xylose in addition to the sugars present in mesquite gum. Hydrolysis of the methylated derivative yields 2 : 3 : 5-trimethyl *l*-arabinose, 2 : 3 : 4-trimethyl *d*-xylose, 2 : 3-dimethyl *l*-arabinose, 2 : 4 : 6-trimethyl *d*-galactose, 2 : 4-dimethyl *d*-galactose, 2-methyl *d*-galactose, and 4-methyl *d*-galactose, as well as 2 : 3-dimethyl and 2 : 3 : 4-trimethyl glucuronic acid. It is, therefore, much more complicated in structure than is mesquite gum; it also is a polymer of the branched chain type and similarly contains end groups of *l*-arabofuranose and *d*-glucuronic acid residues, as well as units of *d*-galactopyranose as a part of the molecule.

Whether a polysaccharide is homogeneous or heterogeneous is one of the major difficulties encountered in work on these highly polymeric materials. For example, work on yeast mannan has shown that the polysaccharide has a ramified structure. This result has been confirmed<sup>10</sup> and extended by W. N. Haworth, R. L. Heath, and S. Peat.<sup>11</sup> According to R. Garzuly-Janke,<sup>12</sup> however, yeast mannan is a mixture of three mannans. Similarly, salep-mannan<sup>13</sup> was shown to be an unbranched polymeric material of a very heterogeneous nature. B. Drake<sup>14</sup> is of the opinion that lichenin is a polymer built up of glucose residues linked through C<sub>1</sub> and C<sub>4</sub> only. K. Meyer and P. Gürtler,<sup>15</sup> however, consider that it contains some 10–20% of  $\beta$ -1 : 3-linkages, no 1 : 6-linkages being present. *iso*Lichenin is a mixture of polysaccharides and on hydrolysis yields galactose, glucose, and mannose. Pustulin<sup>16</sup> from *Umbilicaria pustulata* resembles barley root glucosan<sup>17</sup> in that it is a 1 : 6-polyglucan, while yeast glucan<sup>18</sup> like laminarin<sup>42b</sup> is a polymer consisting of glucose residues linked through the hydroxyl groups on C<sub>1</sub> and C<sub>3</sub>, since it is unaffected by periodic acid. Little is known of the fine structure of these polysaccharides.

The increasing industrial importance of pectin has stimulated research on the physical properties and chemical reactions of pectins and their derivatives. For example, pectin has been used as a source of *d*-galacturonic acid, 5-keto-*l*-galactonic acid, vitamin C, and *l*(+)-tartaric acid.<sup>19</sup> Methods have been described for the acylation of pectin<sup>20</sup> using formamide or acetone-pyridine as dispersing agents. The thermal degradation of pectin solution<sup>21</sup>

<sup>10</sup> G. Lindstedt, *Arkiv Kemi, Min. Geol.* 1945, **20** A, No. 13.

<sup>11</sup> *J.*, 1941, 833.

<sup>12</sup> *J. pr. Chem.*, 1940 [ii], **156**, 45.

<sup>13</sup> E. Huseman, *ibid.*, 1940 [ii], **155**, 246. <sup>14</sup> *Biochem. Z.*, 1943, **313**, 388.

<sup>15</sup> *Arch. Sci. phys. nat.*, 1945, **27**, V, Suppl. 97.

<sup>16</sup> V. C. Barry and T. Dillon, *Proc. Roy. Irish Acad.*, 1943, **49**, B, 177.

<sup>17</sup> W. Z. Hassid, *J. Amer. Chem. Soc.*, 1939, **61**, 1224.

<sup>18</sup> W. Z. Hassid, M. A. Joslyn, and R. M. McCready, *ibid.*, 1941, **63**, 295.

<sup>19</sup> U.S.P.P. 2,338,534, 2,338,115; H. S. Isbell and Nancy B. Holt, *J. Res. Nat. Bur. Stand.*, 1945, **35**, 433.

<sup>20</sup> G. Schneider and M. Ziervogel, *Ber.*, 1936, **69**, 2530; J. F. Carson, jr., and W. D. Maclay, *J. Amer. Chem. Soc.*, 1945, **67**, 787; 1946, **68**, 1015.

<sup>21</sup> R. C. Merrish and M. Weeks, *ibid.*, 1945, **67**, 2244.



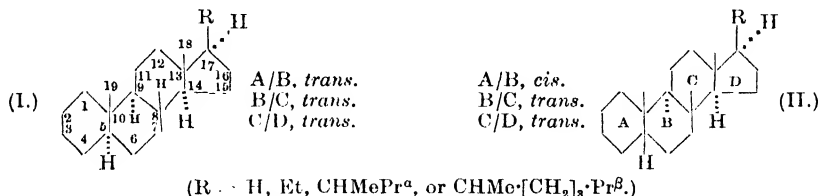
as determined by loss of viscosity has been shown to be due to breaking of glycosidic links and not to hydrolysis of the ester grouping, but this loss of viscosity in some cases may be due rather to an atmospheric oxidation of the pectin molecule, which is catalysed by traces of ascorbic acid.<sup>22</sup> Tamarind seed pectin<sup>23</sup> differs from most pectins in being built up of xylose, glucose, and galactose residues, while the polyuronide fraction of New Zealand flax<sup>24</sup> is not a pectin, such as appears to be present in some varieties of flax, but a hemicellulose-like material built up of *d*-xylose and *d*-glucuronic acid residues. The capsular polysaccharide of *Rhizobium radicicolum*<sup>25</sup> consists of  $\beta$ -linked *d*-glucose and *d*-glucuronic acid residues and appears to be similar to cellulose with the  $\text{CH}_2\text{OH}$  group oxidised to  $-\text{CO}_2\text{H}$  on alternate sugar units, and in this respect resembles some of the bacterial capsular polysaccharides.

J. K. N. J.

### 5. STEROIDS AND RELATED COMPOUNDS.

Steroid stereochemistry was last reviewed in 1938;<sup>1</sup> subsequent observations<sup>2</sup> have supported the suggestions of Ruzicka<sup>3</sup> as to the configuration of the steroid nucleus, but recent modification of the orientations assigned to nuclear substituents, *e.g.*, the  $\text{C}_{17}$ -side chain, have had wide repercussions.

*Configuration of the Steroid Nucleus.*—The nuclear structure of androstane, 5-*allopregnane*, 5-*allocholane*, and *cholestane* is commonly represented by (I), and that of *etiocholane*, *pregnane*, *cholane*, and *coprostanane* by (II).



(Throughout this report, the angular methyl groups attached to  $\text{C}_{10}$  and  $\text{C}_{13}$  will be represented by strokes.)

*trans*-Fusion of rings A/B and C/D in (I) indicates that the angular methyl groups attached to  $\text{C}_{10}$  and  $\text{C}_{13}$  are similarly orientated and they are by convention regarded as projecting forward from the plane of the

<sup>22</sup> H. Douel, *Helv. Chim. Acta*, 1943, **26**, 2002.

<sup>23</sup> T. P. Ghose, S. Krishna, and P. S. Rao, *J. Sci. Ind. Res. India*, 1946, **4**, 705.

<sup>24</sup> J. Mellroy, G. S. Holmes, and (Miss) R. P. Mauger, *J.*, 1945, 796.

<sup>25</sup> (Miss) M. Schluchterer and M. Stacey, *J.*, 1945, 776.

<sup>1</sup> R. K. Callow, *Ann. Reports*, 1938, **35**, 281.

<sup>2</sup> G. Giacomello, *Gazzetta*, 1939, **69**, 790; J. D. Bernal, D. Crowfoot, and I. Fankuchen, *Proc. Roy. Soc.*, 1940, **A**, **239**, 135; K. Dimroth and H. Jonsson, *Ber.*, 1941, **74**, 520; C. H. Carlisle and D. Crowfoot, *Proc. Roy. Soc.*, 1945, **A**, **184**, 64; W. D. Kumler, *J. Amer. Chem. Soc.*, 1945, **67**, 1901.

<sup>3</sup> L. Ruzicka, M. Furter, and G. Thomann, *Helv. Chim. Acta*, 1933, **16**, 331.

paper, *i.e.*, as ( $\beta$ )-orientated.\* This condition also holds for (II), since, for example, enolisation of 6-keto-derivatives of (II) leads to 6-keto-derivatives of (I).<sup>6</sup> However, the absolute configuration of no single steroid centre of asymmetry has yet been determined, despite the contrary statement of E. Bergmann,<sup>7</sup> so that the stereochemical arrangements of androstane and  $\alpha$ -etiocholanone may actually be the mirror images of formulæ (I) and (II) respectively.

The chair form of *cyclohexane* is a rigid structure; it might therefore be thought that (I) is the rigid structure depicted in Fig. 1. Ring A can, however, undergo conversion into a boat form, by relative motion of C<sub>2</sub>, C<sub>3</sub>, and C<sub>4</sub> whereby C<sub>3</sub> and C<sub>10</sub> become the ends of the boat, without disturbance of the remainder of the tetracyclic structure.<sup>8</sup> The *cis*-union of rings A/B in (II) suggests that rings A and B are both boat forms<sup>3</sup> (Fig. 2), which at first sight preserves the lath-shaped type characteristic of molecules based on (I). The boat form of *cyclohexane* is, however, a mobile structure, and in (II) rings A and B are capable of crumpling to an astonishing degree in spite of the fact that B is partly locked by *trans*-fusion with C; that such crumpling does occur is shown by the remarkable series of 3:9-epoxy-compounds described by Kendall *et al.*<sup>9</sup> Another possible modification of

\* L. Fieser, "The Chemistry of Natural Products Related to Phenanthrene", 2nd Edition, New York, 1937, pp. 398, 399; compare R. K. Callow, *ref.* (1).

<sup>5</sup> T. Reichstein and C. W. Shoppee, "Vitamins and Hormones", New York, 1943, p. 349.

<sup>6</sup> V. Prelog and E. Tagmann, *Helv. Chim. Acta*, 1944, **27**, 1880; A. Windaus, *Annalen*, 1926, **447**, 233.

<sup>7</sup> *J. Soc. Chem. Ind.*, 1939, **58**, 512.

<sup>8</sup> C. W. Shoppee, *J.*, 1946, 1138.

<sup>9</sup> V. R. Mattox, R. B. Turner, L. L. Engle, B. F. McKenzie, W. F. McGuckin, and E. C. Kendall, *J. Biol. Chem.*, 1946, **164**, 569; **166**, 345.

\* The convention adopted for the representation and description of substituted steroids is that proposed by Fieser,<sup>4</sup> and extended by Reichstein and Shoppee;<sup>5</sup> position is specified by the number of the nuclear carbon atom bearing the substituent, configuration by the suffix ( $\alpha$ ) or ( $\beta$ ). As emphasised by Callow,<sup>1</sup> the parentheses are important for a double reason; they not only distinguish from trivial indices, " $\alpha$ " and " $\beta$ ", but also indicate a definite stereochemical orientation. Thus the position and configuration of the hydroxyl group common to cholesterol, dihydrocholesterol, and coprostanol (coprosterol) are defined by the expression 3( $\beta$ ); this hydroxyl group projects forward from the plane of the paper as does the C<sub>10</sub>-angular methyl group, and this is shown by a full-line bond. In *epicholesterol*, *epidihydrocholesterol*, and *epicoprostanol* (*epicoprosterol*) the position and configuration of the hydroxyl group is defined by the expression 3( $\alpha$ ); this hydroxyl lies below the plane of the paper, and this is shown by a broken-line bond.

For substituents in steroid side chains, *e.g.*, secondary or tertiary but not primary hydroxyl groups, position is specified by the number of the carbon atom bearing the group in question, but, since stereoisomerism is now no longer geometrical in character but of the classical tartaric acid type, a definite spatial orientation cannot in general be assigned. The indices  $\alpha$  and  $\beta$  without parentheses, adjacent to and following a position numeral, *e.g.*, 20 $\alpha$ , 20 $\beta$ , should be employed, but *n*- and *iso*- have also been used. These suffixes without parentheses serve solely to distinguish stereoisomerides without any spatial implication; and since here suffix assignment is arbitrary, compounds labelled, *e.g.*, 20 $\beta$ , will not necessarily have corresponding configuration at C<sub>20</sub>.

(II), based on a structure proposed for *cis*-decalin by Bastiansen and Hassell,<sup>10</sup> is shown in Fig. 3; despite *cis*-union both rings A and B are chair forms, and the molecule is L-shaped.\* Ring A of the structure shown in Fig. 3 can also become a boat form with ends at C<sub>3</sub> and C<sub>10</sub> by relative motion of C<sub>2</sub>, C<sub>3</sub>, and C<sub>4</sub>, the L-shape being preserved. Existing X-ray evidence

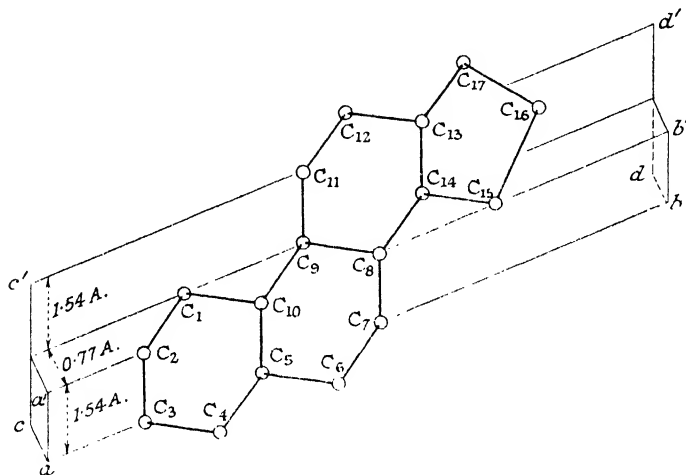


FIG. 1.

Carbon skeleton of androstane with rings A and B as chair forms.

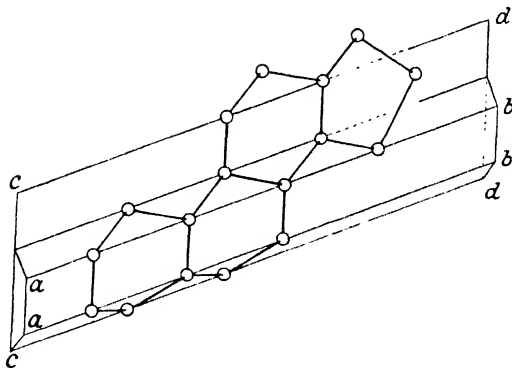


FIG. 2.

Carbon skeleton of atiocholane with rings A and B as boat forms.

for coprostan compounds<sup>2</sup> suggests that these are flat and not L-shaped as in Fig. 3, although on energetic grounds the chair structure of Fig. 3

<sup>10</sup> O. Bastiansen and O. Hassell, *Nature*, 1946, **157**, 765; the legend to Fig. 1 in this communication is incorrect: (a) shows the structure of *trans*-decalin according to Mohr, (b) that of *cis*-decalin according to the authors.

\* Compare L. Ruzicka, M. Furter, and M. W. Goldberg, *Helv. Chim. Acta*, 1938, **21**, 498, Plate II.

would be expected to be more stable than the boat structure of Fig. 2, because, on account of the greater separation of the hydrogen atoms in a chair form, the mutual repulsions of the electrons involved in the C-H bonds are less. Shoppee<sup>8</sup> has calculated the height of the energy barrier of the chair  $\rightarrow$  boat conversion in *cyclohexane* as  $\sim 10$  kg.-cals.; chair-boat transformations between the types indicated above might be expected to involve energies of the same order, which are small compared with the activation energies of most chemical reactions and appear to be derivable from thermal bombardment at ordinary temperatures. Stereochemistry deals with structures which retain individuality under such conditions; from the stereo-

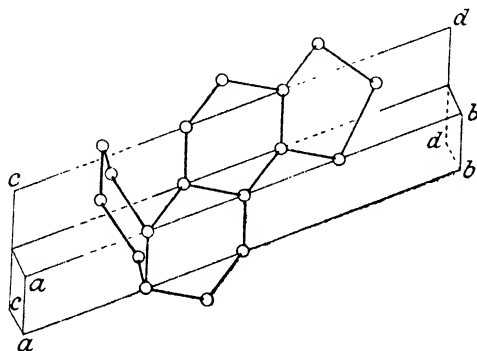


FIG. 3.

*Possible nuclear structure of ætiocholane with rings A and B as chair forms.*

*(All seen in isometric projection at 22.5°.)*

chemical point of view it is useless to attempt to distinguish between geometrical arrangements which are separated by energy hills so low that they will normally be traversed with great frequency in the liquid state or in solution. Very many substitution products of androstane, ætiocholane, and their homologues are known, but there is no established case of the existence of more than the two epimeric substitution products predicted on the basis of formulæ (I) and (II). The various possible geometrical modifications of (I) and (II) thus appear to make no contribution to the stereochemistry of these substances and their derivatives. Such geometrical modifications cannot affect the character (*cis*- or *trans*-) of a ring-fusion already present in a molecule.

*Configuration of Nuclear Substituents.*—Four methods have been used to determine the configuration of nuclear substituents :

(i) *Formation of a cyclic compound.* If two substituents at non-adjacent carbon atoms interact to give a cyclic derivative, *e.g.*, a lactone<sup>11</sup> or an oxide,<sup>9</sup> they must lie on the same side of the general plane of the ring system,<sup>12</sup> *i.e.*, must both be ( $\alpha$ )- or ( $\beta$ )-orientated.

<sup>11</sup> H. Lettré, *Ber.*, 1935, **68**, 766.

<sup>12</sup> K. Alder and G. Stein, *Annalen*, 1933, **504**, 229.

(ii) *Examination of reaction mechanism.* On the basis of the geometrical form and energy of the transition state, it has been concluded that for the bimolecular replacement ( $S_N2$ ) of one substituent by another at positions 1—4, 6, 7, 11, 12, and probably 15—17 in androstane (I), inversion should be the rule;<sup>8</sup> a similar conclusion may be expected to hold for derivatives of ætiocholane (II). Conversely, in the presence of suitably placed unsaturated centres, *e.g.*, in derivatives of androstene (and probably also ætiocholene), retention of configuration should be the rule, the substitution mechanism involved here probably being that designated  $S_N1$ .<sup>13</sup>

(iii) *Examination of reaction rates.* Since in bimolecular substitutions ( $S_N2$ ) steric effects operate,<sup>14</sup> proximity to an angular methyl group of a ( $\beta$ )-orientated substituent, *e.g.*, at  $C_{11}$  or  $C_{17}$ , as compared with the same ( $\alpha$ )-orientated substituent should and does cause a marked difference in reaction velocity.<sup>15, 16, 17</sup> Interpretation of such rate-differences requires consideration of the stereochemical character of the reaction. Thus, alkaline hydrolysis proceeds more rapidly for an 11( $\beta$ )-bromo-steroid than for the 11( $\alpha$ )-epimeride, but more slowly for a 17( $\beta$ )-acetoxy-steroid than for the 17( $\alpha$ )-epimeride; both reactions occur predominantly by mechanism  $S_N2$ , but the former proceeds with inversion of configuration at  $C_{11}$  and the latter with preservation of configuration at  $C_{17}$ . Since formation of the transition state is the rate-determining stage, ease of entry of the attacking hydroxyl ion to the relatively sterically unhindered ( $\alpha$ )-face of  $C_{11}$  in the 11( $\beta$ )-bromo-compound is determinative, and the rates are 11( $\beta$ )-Br  $\gg$  11( $\alpha$ )-Br.<sup>17</sup> In the case of a 17-acetoxyl group, replacement occurs not at  $C_{17}$  but at the carbonyl carbon atom of the acetyl group, which is already ( $\alpha$ )- or ( $\beta$ )-orientated to the general plane of the ring system; ease of approach of the attacking hydroxyl ion is again determinative, but, because only the 17( $\beta$ )-acetoxy group can be hindered by the angular methyl group at  $C_{13}$ , the rates are 17( $\alpha$ )-OAc  $>$  17( $\beta$ )-OAc.<sup>15</sup>

(iv) *Application of optical superposition rules.*<sup>8, 18, 19, 20, 21, 22</sup> Examples of these four methods will be encountered in the following review of the various nuclear positions, which makes no claim to be complete.

*Positions  $C_1$ ,  $C_2$ , and  $C_4$ .*—Catalytic hydrogenation with platinum in an acidic or neutral medium of 2-acetoxycholestan-3-one gives a complex mixture from which, unexpectedly, the acetate of a 1-hydroxycholestan-3-one is isolated;<sup>23</sup> the configuration of the 1-hydroxyl group is unknown, but the

<sup>13</sup> C. W. Shoppee, *J.*, 1946, 1147.

<sup>14</sup> I. Dostrovsky, E. D. Hughes, and C. K. Ingold, *ibid.*, p. 173.

<sup>15</sup> L. Ruzicka, M. Furter, and M. W. Goldberg, *Helv. Chim. Acta*, 1938, **21**, 498.

<sup>16</sup> B. Koechlin and T. Reichstein, *ibid.*, 1942, **25**, 919.

<sup>17</sup> T. F. Gallagher and W. P. Long, *J. Biol. Chem.*, 1946, **162**, 521, 533.

<sup>18</sup> R. K. Callow and F. G. Young, *Proc. Roy. Soc.*, 1936, **157**, A, 194.

<sup>19</sup> S. Bernstein, W. Kauzmann, and E. S. Wallis, *J. Org. Chem.*, 1941, **6**, 319.

<sup>20</sup> S. Bernstein, E. J. Wilson, junr., and E. S. Wallis, *ibid.*, 1942, **7**, 103.

<sup>21</sup> P. A. Plattner and H. Heusser, *Helv. Chim. Acta*, 1944, **27**, 748.

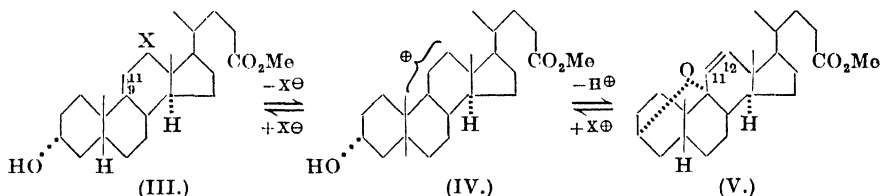
<sup>22</sup> D. H. R. Barton, *J.*, 1945, 813; 1946, 512.

<sup>23</sup> L. Ruzicka, P. A. Plattner, and M. Furrer, *Helv. Chim. Acta*, 1944, **27**, 524, 727.

compound gives no precipitate with digitonin, and by oxidation yields cholestan-1-one. Also formed in the reduction are a 2:3-dihydroxycholestan-2<sup>24</sup> and the monoacetates of three diols, which may be stereoisomerides. A mixture of stereoisomeric 2:3-dihydroxycholestanes is also obtained by oxidation of cholest-4-en-3-one with lead tetra-acetate followed by hydrogenation with platinum-acetic acid.<sup>25</sup> Wolff-Kishner reduction of 2-acetoxycholestan-3-one gives, in addition to cholestan-2-one and the normal product 2-hydroxycholestan-2-one (isolated as cholestan-2-one), the above 1-hydroxycholestan-2-one and 4(β)-hydroxycholestan-2-one;<sup>23</sup> by contrast, Wolff-Kishner reduction of 3-acetoxycholestan-3-en-2-one<sup>26</sup> gives only cholestan-2-one. The production from 2-acetoxycholestan-3-one with alkalis of the 4-oxygenated compounds, 3:4-dihydroxycholestan-3-one and 3-hydroxycholestan-4-one, again indicates the surprising lability of the 2-hydroxyl group. A 2-bromine atom also exhibits similar lability; whereas 2-bromocholestan-3-one with hot collidine gives the expected cholest-1-en-3-one, use of potassium acetate in acetic acid yields exclusively a "hetero-Δ<sup>1</sup>-ketone" now shown to be cholest-5-en-4-one.<sup>27</sup> Whether such lability\* is connected with the steric orientation of the 2-substituent is not known.

Cholestan-2-one is reduced with platinum-acetic acid to 2(β)-hydroxycholestan-2-one (precipitated by digitonin) and by sodium-ethanol to 2(α)-hydroxycholestan-2-one.<sup>23</sup>

*Position C<sub>3</sub>.*—Proof that the 3-hydroxyl group of the bile acids is (α)-orientated has been adduced by Kendall *et al.*<sup>9</sup> In anhydrous non-polar



solvents methyl 3(α)-hydroxy-12-halogeno-chole-9(11)-en-2-ate (III; X = Cl or Br) is stable, but the halogen is completely removed in a few minutes when

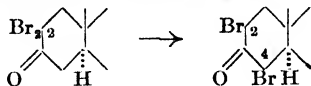
<sup>24</sup> R. E. Marker and L. Plambeck, junr., *J. Amer. Chem. Soc.*, 1939, **61**, 1332.

<sup>25</sup> E. Seebeck and T. Reichstein, *Helv. Chim. Acta*, 1944, **27**, 948; cf. G. Ehrhart, H. Rushig, and W. Aumüller, *Ber.*, 1939, **72**, 2035.

<sup>26</sup> E. T. Stiller and O. Rosenheim, *J.*, 1938, 353.

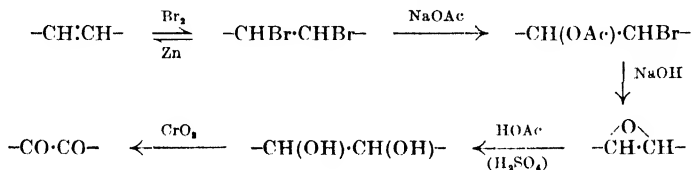
<sup>27</sup> A. Butenandt and G. Ruhenstroth-Bauer, *Ber.*, 1944, **77**, 397; cf. A. Butenandt *et al.*, *ibid.*, 1935, **68**, 1850, 1854, 2091; 1936, **69**, 1158; 1938, **71**, 1681; 1940, **73**, 206; cf. also L. Ruzicka, P. A. Plattner, and R. Aeschbacher, *Helv. Chim. Acta*, 1938, **21**, 866.

\* Since this report was written a further example,



constituting the essential step in the production of the key-intermediate in the synthesis of "α"-cestradiol from cholesterol (H. H. Inhoffen and G. Zühlsdorff, *Ber.*, 1941, **74**, 1911; 1943, **76**, 233) has been described (A. L. Wilds and C. Djerassi, *J. Amer. Chem. Soc.*, 1946, **68**, 2125).

a chloroform solution is washed with water at 20° to give, *via* the mesomeric cation (IV), the 3:9-epoxide (V). By contrast the acetate of (III) is entirely stable under these conditions, and affords a diene of unspecified structure only with boiling pyridine. The 3:9-epoxy-structure survives the following sequence of reactions at the C<sub>11</sub>:C<sub>12</sub>-double bond:

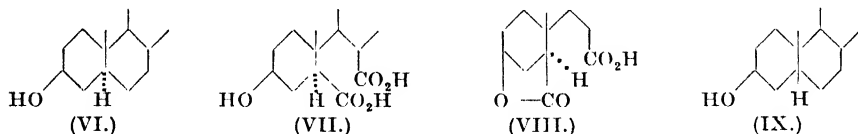


but with hydrochloric or hydrobromic acid undergoes fission to regenerate (III).

There is as yet no strict proof that the hydroxyl group in cholesterol and dihydrocholesterol is ( $\beta$ )-orientated.\* The evidence of X-ray crystallographic analysis is suggestive; the "molecular lengths" (calculated from the slope of the  $\gamma$ -optic axis to the  $c$  plane in the crystals) are:<sup>28</sup>

Cholesterol, 2H <sub>2</sub> O .....	39.5 Å.	<i>epi</i> Cholesterol .....	34.5 Å.
<i>iso</i> Androsterone .....	12.8 Å. †	Androsterone .....	11.25 Å.

so that, as models suggest, a 3( $\beta$ )-hydroxyl group does appear to increase the "molecular length". The proof adduced by Lettré<sup>11</sup> on the basis that the acid (VII) affords a lactone (VIII) is vitiated by lack of knowledge concerning the occurrence or not of Walden inversion at several stages in the conversion of dihydrocholesterol (VI) into the acid (VII).



Measurements of the rate of hydrolysis of 3-epimeric acyloxy-compounds<sup>15</sup> are, however, consistent with the generally accepted structure of dihydrocholesterol as 3( $\beta$ )-hydroxycholestane (VI). The 3-hydroxyl group in coprostanol (coprosterol) has been proved<sup>29</sup> to possess the same configuration as that in (VI) so that, as previously supposed on the basis of Ruzicka's application<sup>30</sup> of the Auwers-Skita rule, coprostanol is 3( $\beta$ )-hydroxy-coprostanol (IX).

The configuration of a 3-hydroxyl group is concerned not only in digitonide

<sup>28</sup> J. D. Bernal, D. Crowfoot, and I. Fankuchen, *Phil. Trans.*, 1940, **239**, 170; J. D. Bernal and D. Crowfoot, private communication.

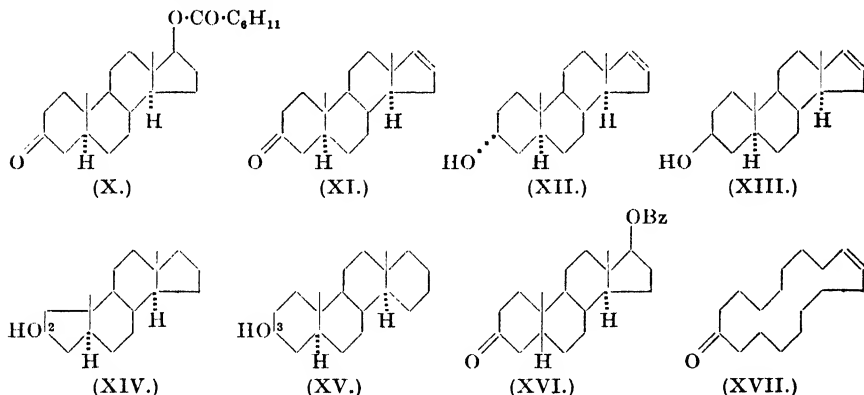
<sup>29</sup> A. Lardon and T. Reichstein, *Helv. Chim. Acta*, 1941, **24**, 955.

<sup>30</sup> L. Ruzicka, H. Brungger, E. Eichenberger, and J. Meyer, *ibid.*, 1934, **17**, 1407.

\* Since this report was written, such a proof has been adduced by the writer by the direct conversion of cholesterol, as the acetate, into the acid (VII).

† The "molecular length" cannot here directly be calculated, but is estimated from consideration, *inter alia*, of strong reflexions combined with the cell dimensions.

precipitation and androgenic activity, but also appears to influence odour. Although steroids in general are odourless, a limited number of androstane derivatives have odours; 3( $\alpha$ )-hydroxy-compounds have much more intense odours than the 3( $\beta$ )-epimerides. 3( $\alpha$ )-Hydroxy- (XII) and 3( $\beta$ )-hydroxy-androst-16-ene (XIII), isolated from pig testicles<sup>31</sup> and synthesised<sup>32</sup> from androstan-17( $\beta$ )-ol-3-one hexahydrobenzoate (X) by pyrolysis and reduction of the resulting ketone (XI) with aluminium *isopropoxide*, have intense and faint musk-like odours respectively. Similar differences are observed between the epimeric saturated analogues,<sup>32, 33, 34</sup> the epimeric 2( $\alpha$ )- and 2( $\beta$ )-hydroxy-*A*-norandrostanes (XIV),<sup>35</sup> and the epimeric 3( $\alpha$ )- and 3( $\beta$ )-hydroxy-*D*-homoandrostanes (XV).<sup>35</sup> The analogous  $\alpha$ tiocholene and  $\alpha$ tiocholane<sup>33, 34, 35, 36</sup> derivatives obtained from  $\alpha$ tiocholan-17( $\beta$ )-ol-3-one benzoate (XVI) are nearly odourless. Incidentally, various ketones of both the androstane and  $\alpha$ tiocholane series, *e.g.*, (XI), have an intense urine-like odour and exhibit a curious structural resemblance to the natural sexual odoriferous compound civetone (XVII).<sup>37</sup>



The constitutions of the so-called " $\alpha$ "- and " $\beta$ "-cholestanyl chlorides have been established as 3( $\beta$ )-chloro-(XIX) and 3( $\alpha$ )-chloro-cholestane (XX) respectively.<sup>8</sup> On the basis of extensive kinetic studies it has been found<sup>38</sup> that in homogeneous substitutions of  $\cdot\text{Hal}$  by  $\cdot\text{OR}$  in alkyl halides containing, besides the halogen, only neutral, saturated groups at the seat of substitution, (a) the predominating orientation is inversion of configuration, no matter whether the mechanism is  $S_N1$  or  $S_N2$ , (b) there is practically complete absence of racemisation in mechanism  $S_N2$ , whilst extensive

<sup>31</sup> V. Prelog and L. Ruzicka, *Helv. Chim. Acta*, 1944, **27**, 61.

<sup>32</sup> V. Prelog, L. Ruzicka, and P. Wieland, *ibid.*, p. 66.

<sup>33</sup> R. D. H. Heard and A. F. McKay, *J. Biol. Chem.*, 1946, **165**, 677.

<sup>34</sup> A. Butenandt and L. A. Suranyi, *Ber.*, 1942, **75**, 591.

<sup>35</sup> V. Prelog, L. Ruzicka, and P. Meister, *Helv. Chim. Acta*, 1945, **28**, 1651.

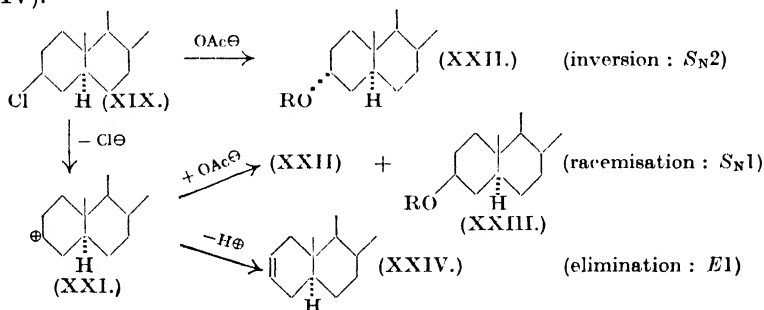
<sup>36</sup> V. Prelog, L. Ruzicka, P. Wieland, and P. Meister, *ibid.*, p. 618.

<sup>37</sup> L. Ruzicka, *ibid.*, 1926, **9**, 230.

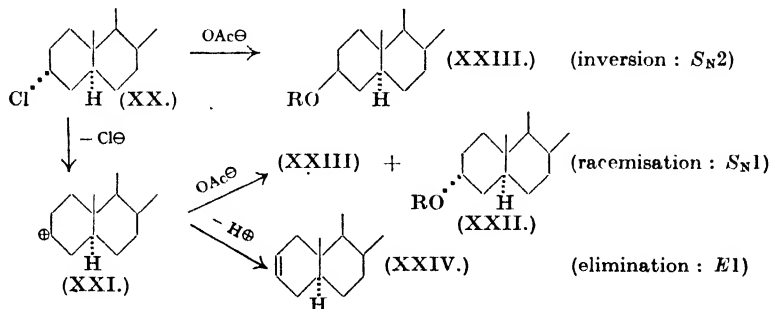
<sup>38</sup> W. A. Cowdrey, E. D. Hughes, C. K. Ingold, S. Mastermann, and A. D. Scott *J.*, 1937, 1259.



racemisation is characteristic of mechanism  $S_N1$ . The cholestanyl chlorides are typical if complex alkyl halides; and the " $\alpha$ "-chloride, m. p. 115°, undergoes homogeneous reaction with acetate ions at 180° to give, after hydrolysis, 3( $\alpha$ )-hydroxycholestane<sup>39</sup> (XXII; R = H); the so-called " $\alpha$ "-cholestanyl chloride is therefore 3( $\beta$ )-chlorocholestane (XIX). Chromatographic analysis makes possible the isolation also of a relatively small quantity of 3( $\beta$ )-hydroxycholestane (XXIII; R = H), and of much cholest-2-ene (XXIV).<sup>8</sup>



The 3( $\alpha$ )-hydroxycholestane (XXII; R = H) arises as the acetate (XXII; R = Ac) principally by mechanism  $S_N2$  with inversion of configuration, and the " $\alpha$ "-chloride is therefore 3( $\beta$ )-chlorocholestane (XIX). The small quantity of 3( $\beta$ )-hydroxycholestane (XXIII; R = H) formed results from the operation of mechanism  $S_N1$ , since the tendency of the intermediate cation (XXI) to become flattened is responsible for racemisation; an equivalent quantity of the 3( $\alpha$ )-epimeride (XXII; R = H) is derived by this mechanism. The cation (XXI), instead of combining with an acetate ion, can eject a proton to undergo the elimination reaction  $E1$ , giving rise to cholest-2-ene (XXIV). " $\beta$ "-Cholestanyl chloride, m. p. 105°, similarly affords the same three products (XXII; R = H), (XXIII; R = H), and (XXIV), but now the proportion of 3( $\beta$ )-hydroxycholestane (XXIII; R = H) largely exceeds that of the 3( $\alpha$ )-epimeride :

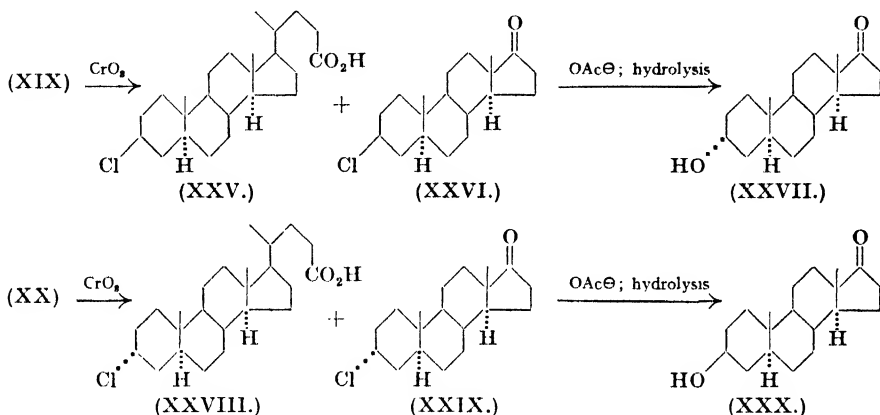


The so-called " $\beta$ "-chloride is therefore 3( $\alpha$ )-chlorocholestane (XX).

<sup>39</sup> R. E. Marker, F. C. Whitmore, and O. Kamm, *J. Amer. Chem. Soc.*, 1935, **57**, 2358.

Similarly, the " $\alpha$ "- and " $\beta$ "-stigmastanyl chlorides<sup>40</sup> which afford respectively 3( $\alpha$ )- and 3( $\beta$ )-hydroxystigmastane are to be regarded as 3( $\beta$ )- and 3( $\alpha$ )-chlorostigmastane, whilst the single ergostanyl chloride<sup>41</sup> known is probably 3( $\alpha$ )-chloroergostane.

Following the establishment of the configurations of the cholestanyl chlorides (XIX) and (XX), it becomes possible to assign configurations to their oxidative degradation products (XXV) and (XXVIII), and to obtain a consistent picture of the conversion of the 3-chloro-ketones (XXVI), and (XXIX), by mechanism  $S_N2$  with inversion, into androsterone (XXVII) and *iso*androsterone (XXX) respectively.



Both chloro-ketones (XXVI, XXIX) undergo the elimination reaction *E1* to give androst-2(?) [or 3(?)]-en-17-one to an extent dependent upon the conditions.<sup>8, 42</sup>

Application<sup>8</sup> of the orientation rules laid down by Hughes, Ingold *et al.*<sup>14, 38</sup> for the replacement of  $\cdot\text{OR}$  by  $\cdot\text{Cl}$  to the 3-hydroxycholestanes (XXII, XXIII;  $\text{R} = \text{H}$ ), the 3-hydroxystigmastanes, androsterone (XXVII) and *iso*androsterone (XXX) furnishes a picture which is consistent with that obtained for replacement of  $\cdot\text{Cl}$  by  $\cdot\text{OR}$ ; in brief, use of thionyl chloride preserves configuration whilst use of phosphorus pentachloride causes inversion.

The establishment of the constitution of the " $\alpha$ "-cholestanyl chloride as (XIX) fixes that of cholesteryl chloride as 3( $\beta$ )-chlorocholest-5-ene<sup>13</sup> (XXXII), because catalytic hydrogenation of (XXXII) gives (XIX) quantitatively; that the 3-chlorine atoms in (XIX) and (XXXII) have the same orientation is also clear from crystallographic evidence.<sup>43</sup> Cholesteryl chloride (XXXII) is obtained from cholesterol (XXXI) by treatment not

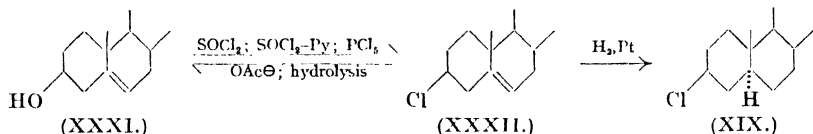
<sup>40</sup> R. E. Marker and E. J. Lawson, *J. Amer. Chem. Soc.*, 1937, **59**, 2711.

<sup>41</sup> F. Reindel and E. Walter, *Annalen*, 1928, **450**, 212; I. M. Heilbron, K. M. Samant, and J. C. E. Simpson, *J.*, 1933, 1410.

<sup>42</sup> A. Butenandt and H. Dannenbaum, *Z. physiol. Chem.*, 1934, **229**, 192.

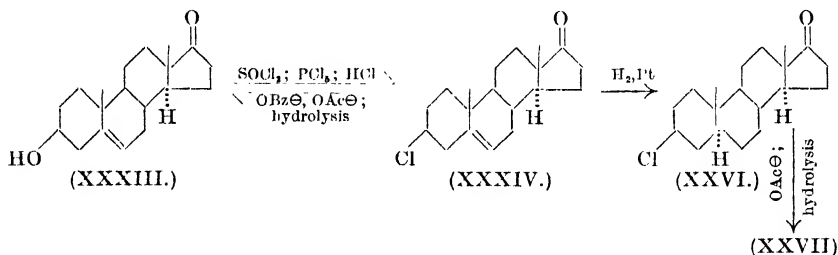
<sup>43</sup> D. Crowfoot, "Vitamins and Hormones," II, New York, 1944, p. 450.

only with thionyl chloride,<sup>44</sup> but also with thionyl chloride-pyridine<sup>45</sup> or phosphorus pentachloride.<sup>46</sup> Thus in contrast to the behaviour of saturated 3-hydroxy-steroids, the hydroxyl group of cholesterol undergoes replacement by chlorine, irrespectively of the reagent employed, with retention of configuration. This singular behaviour reappears in the replacement of  $\cdot\text{Cl}$  by  $\cdot\text{OR}$ , for when cholesteryl chloride (XXXII) is treated with acetate



ions at  $100^\circ$  the product is practically pure cholesteryl acetate and hydrolysis gives a 91% overall yield of cholesterol (XXXI) unaccompanied by *epi*-cholesterol,<sup>13</sup> i.e., there is complete preservation of configuration in sharp contrast with corresponding reactions of the cholestanyl chlorides. Moreover in the ionic reaction (XXXII  $\longrightarrow$  XXXI) it is difficult to envisage the operation of some internal rearrangement mechanism analogous to  $S_Ni$ .<sup>14, 38</sup> Similar relationships exist between other 3-hydroxy- $\Delta^5$ -steroids and their 3-chloro-derivatives, e.g., stigmaterol<sup>40</sup> and " $\beta$ "-sitosterol.<sup>40</sup>

Cholesteryl chloride (XXXII) is oxidised (as the dibromide) by chromium trioxide to " $\alpha$ "-chloroandrosterone,<sup>47</sup> which therefore must be 3( $\beta$ )-chloro-androst-5-en-17-one (XXXIV), a structure which is confirmed by catalytic hydrogenation<sup>42, 48, 49</sup> to the chloro-ketone (XXVI); (XXXIV) is also formed from dehydroisoandrosterone (XXXIII) with preservation of configuration not only by use of thionyl chloride<sup>49</sup> but also by use of phos-



phorus pentachloride<sup>50</sup> and of hydrochloric acid.<sup>42, 49</sup> In (XXXIV), replacement of  $\cdot\text{Cl}$  or  $\cdot\text{OR}$  using benzoate ions in molten benzoic acid<sup>42</sup>

<sup>44</sup> O. Diels *et al.*, *Ber.*, 1904, **37**, 3092; 1911, **44**, 287.

<sup>45</sup> P. J. Daughenbaugh and J. B. Allison, *J. Amer. Chem. Soc.*, 1929, **51**, 3665; see also ref. 38, p. 1267.

<sup>46</sup> J. Mauthner, *Monatsh.*, 1894, **15**, 87.

<sup>47</sup> R. E. Marker, F. C. Whitmore, O. Kamm, T. S. Oakwood, and S. M. Blatterman, *J. Amer. Chem. Soc.*, 1936, **58**, 338.

<sup>48</sup> E. S. Wallis and E. Bernholz, *ibid.*, 1935, **57**, 1379, 1504.

<sup>49</sup> A. Butenandt, H. Dannenbaum, G. Hanisch, and H. Kudzus, *Z. physiol. Chem.*, 1935, **237**, 57.

<sup>50</sup> E. S. Wallis and E. Fernholz, *J. Amer. Chem. Soc.*, 1937, **59**, 764.

or acetate ions in acetic acid also occurs with retention of configuration to yield dehydroisandrosterone benzoate or acetate, and, after hydrolysis, dehydroisandrosterone (XXXIII). Again there is sharp contrast with the saturated series, since the chloro-ketone (XXVI) by similar treatment affords androsterone (XXVII) with inversion.

It has been shown<sup>51</sup> by a kinetically controlled stereochemical examination that the first order hydroxylation and methoxylation of the  $\alpha$ -bromopropionate ion take place with 90–100% retention of configuration and without detectable racemisation, whilst a case of substitution involving an intermediate cation preserved as to configuration by an  $\alpha$ -bromine atom has been reported.<sup>52</sup> It is suggested<sup>13</sup> that the uniform retention of configuration displayed by  $\Delta^5$ -steroids in replacement reactions at  $C_3$  is due to the operation of mechanism  $S_N1$ , the polarisable electrons of the  $\Delta^5$ -unsaturated centre reacting sufficiently powerfully with the positive charge of the intermediate carbon cation to overcome both the energetic and geometrical factors, which normally operate to favour the production of a transition state of linear type the consequence of which is inversion, and leading to the formation of a transition state of pyramidal type, which corresponds to retention of stereochemical form. It is to be expected that suitably placed unsaturated centres may produce similar effects in substitution reactions at positions other than  $C_3$ .

*Positions  $C_5$  and  $C_6$ .*—The structure of the cholestanetriol ("triol I" of Ellis and Petrow<sup>53</sup>) (XXXV) has been confirmed, but the isomeride ("triol II"), previously described as coprostane-3( $\beta$ ):5:6( $\beta$ )-triol, is actually cholestane-3( $\beta$ ):5:6( $\alpha$ )-triol<sup>54</sup> (XXXVI).<sup>\*</sup> Dehydration of the triols (XXXV) and (XXXVI) as the 3:6-diacetates with thionyl chloride in pyridine<sup>55</sup> gives the 3:6-diacetates of the 6-epimerides, (XXXVII) and (XXXVIII) respectively; these by Oppenauer oxidation both yield cholestane-3:6-dione. Earlier statements<sup>56, 57</sup> that the triols (XXXV) and (XXXVI) afford isomerides by oxidation with chromium trioxide are incorrect; both give cholestane-5-ol-3:6-dione.<sup>54, 58</sup>

Hydrogenation of (XXXVII) with platinum in acetic acid<sup>59</sup> leads smoothly to coprostane-3( $\beta$ ):6( $\beta$ )-diol<sup>60</sup> (providing a convenient route to the coprostane series),<sup>54</sup> which by oxidative degradation as the diacetate

<sup>51</sup> W. A. Cowdrey, E. D. Hughes, and C. K. Ingold, *J.*, 1937, 1208.

<sup>52</sup> S. Winstein and H. J. Lucas, *J. Amer. Chem. Soc.*, 1939, **61**, 1576, 2854; S. Winstein and R. E. Buckles, *ibid.*, 1942, **64**, 2780; S. Winstein, *ibid.*, p. 2792.

<sup>53</sup> B. Ellis and V. A. Petrow, *J.*, 1939, 1078.

<sup>54</sup> V. Prelog and E. Tagmann, *Helv. Chim. Acta*, 1944, **27**, 1867.

<sup>55</sup> V. A. Petrow, O. Rosenheim, and W. W. Starling, *J.*, 1938, 679.

<sup>56</sup> A. Windaus, *Ber.*, 1907, **40**, 257.

<sup>57</sup> R. H. Pickard and J. Yates, *J.*, 1908, **93**, 1678.

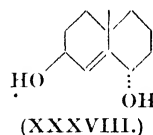
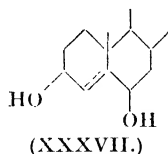
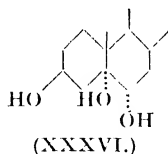
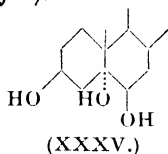
<sup>58</sup> M. Ehrenstein, *J. Org. Chem.*, 1939, **4**, 506.

<sup>59</sup> V. Prelog and E. Tagmann, *Helv. Chim. Acta*, 1944, **27**, 1880.

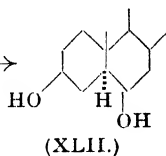
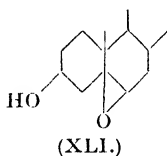
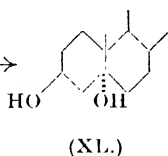
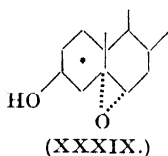
<sup>60</sup> R. E. Marker and J. E. Krueger, *J. Amer. Chem. Soc.*, 1940, **62**, 79.

\* A suffix, ( $\alpha$ ) or ( $\beta$ ), is not essential at  $C_6$  since the orientation of a 5-substituent is already specified by the description cholestane or coprostane.

gives 3( $\beta$ ):6( $\beta$ )-dihydroxyaticholan-17-one and 3( $\beta$ ):6( $\beta$ )-dihydroxycholan-ic acid;<sup>61</sup> this acid is different from " $\beta$ "-hyodeoxycholic acid<sup>62</sup> which is therefore 3( $\beta$ ):6( $\alpha$ )-dihydroxycholan-ic acid. " $\alpha$ "-Hyodeoxycholic acid<sup>63, 64</sup> is a 3( $\alpha$ ):6-dihydroxycholan-ic acid; the configuration at C<sub>6</sub> appears to be ( $\beta$ ) because various workers<sup>60, 64</sup> have been able to achieve partial hydrolysis of 3:6-diacetoxy- to 6-monoacetoxy-compounds (*vide infra*).



The structures of the so-called " $\alpha$ "- and " $\beta$ "-oxides of cholesterol have been established.<sup>65</sup> The " $\alpha$ "-oxide is 5:6( $\alpha$ )-oxidocholestane-3( $\beta$ )-ol (XXXIX); hydrogenation as the acetate<sup>66</sup> gives quantitatively the acetate of cholestane-3( $\beta$ ):5-diol (XL), also obtained directly from the " $\alpha$ "-oxide by use of phenyl-lithium<sup>67</sup> and palladium-acetic acid,<sup>68</sup> whilst fission with



aqueous dioxan at 150° gives the triol (XXXV),<sup>65</sup> with retention of configuration at C<sub>5</sub> and inversion at C<sub>6</sub>. The inclusion of the diol (XL) in the cholestane series is supported by other evidence; thus a diacetate is relatively easily obtained, whereas in a coprostane derivative steric hindrance at C<sub>5</sub> by the C<sub>10</sub> angular methyl group should be more marked. This diacetate is readily hydrolysed to the 5-monoacetate (XLIII); this, by treatment with *p*-toluenesulphonyl chloride in boiling pyridine, gives 70% of epicholesteryl acetate (XLVI), thus affording a remarkable illustration of the intervention, discovered by Winstein *et al.*,<sup>69</sup> of a suitably placed acetyl group in a substitution reaction. The reaction represents an intramolecular S<sub>N</sub>2 substitution; the ( $\alpha$ )-orientation of the 5-acetoxy group (the substituting reagent) together with the ( $\beta$ )-orientation of the 3-tosyl

<sup>61</sup> J. S. Moffatt, *J.*, in the press.

<sup>62</sup> S. Kimura, *Z. physiol. Chem.*, 1937, **248**, 280.

<sup>63</sup> A. Windaus *et al.*, *Annalen*, 1923, **433**, 278; 1926, **447**, 233; *Z. physiol. Chem.*, 1933, **215**, 18.

<sup>64</sup> T. F. Gallagher and J. R. Xenos, *J. Biol. Chem.*, 1946, **165**, 365; W. M. Hoehn, R. B. Moffatt, J. Linsk, and J. E. Stafford, *J. Amer. Chem. Soc.*, 1946, **68**, 1855, 1857.

<sup>65</sup> P. A. Plattner and W. Lang, *Helv. Chim. Acta*, 1944, **27**, 1872.

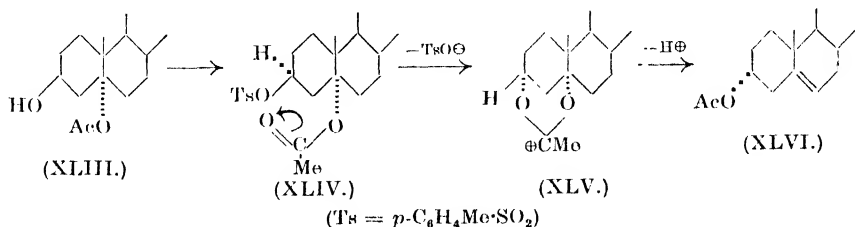
<sup>66</sup> P. A. Plattner, T. Petrzilka, and W. Lang, *ibid.*, p. 573.

<sup>67</sup> A. D. Tschinajewa and M. I. Uschakow, *J. Gen. Chem. Russia*, 1941, **11**, 335.

<sup>68</sup> H. Stavely, *J. Amer. Chem. Soc.*, 1942, **64**, 2723.

<sup>69</sup> S. Winstein, H. V. Hess, and R. E. Buckles, *ibid.*, p. 2796.

group (the group substituted) in (XLIV) necessitates that the transition state must be of linear type, the consequence of which is inversion of configuration in the intermediate cation (XLV).



The products previously described as the “ $\beta$ ”-oxide, “ $\beta$ ”-oxide-acetate, and “ $\beta$ ”-oxide-benzoate of cholesterol<sup>70, 71, 72</sup> have been shown<sup>73</sup> to be molecular compounds; the true “ $\beta$ ”-oxide is obtained from 5-chloro-cholestane-3( $\beta$ ):6( $\beta$ )-diol diacetate with alcoholic potassium hydroxide,<sup>74</sup> or from the molecular compound—termed the “ $\alpha\beta$ ”-oxide-acetate—by fission and separation of the components on a column of aluminium oxide.<sup>66</sup> Hydrogenation of the “ $\beta$ ”-oxide acetate gives cholestane, cholestane-3( $\beta$ )-ol acetate, and the 3-monoacetate of a cholestane-3( $\beta$ ):6-diol.<sup>66</sup> The last named is identified by comparison with the two known cholestane-3( $\beta$ ):6-diols, and configuration is assigned as follows.<sup>66</sup> The diacetate<sup>75, 76</sup> of one of these diols (Windaus) undergoes hydrolysis with dilute potassium hydroxide readily, both acetoxy groups being hydrolysed at similar rates; the diacetate<sup>59, 60</sup> of the other diol (Marker-Prelog) possesses one acetoxy group which is relatively difficult to hydrolyse [a feature also exhibited by the 3:6 diacetate of (XXXV)]; conversely only one hydroxyl group of this diol is easily esterified.<sup>77</sup> Since alkaline hydrolysis should proceed by mechanism  $S_N2$  and so be subject to steric hindrance and because a 6( $\beta$ )-acetoxy-group should be sterically hindered by the  $C_{10}$  angular methyl group\* to a greater extent than a 6( $\alpha$ )-acetoxy-group, the Windaus compound is the 3( $\beta$ ):6( $\alpha$ )-diol, whilst the Marker-Prelog epimeride, which is that obtained by hydrogenation of the “ $\beta$ ”-oxide acetate, must be the 3( $\beta$ ):6( $\beta$ )-diol (XLII). The “ $\beta$ ”-oxide of cholesterol is therefore 5:6( $\beta$ )-oxido-coprostan-3( $\beta$ )-ol (XLI). Hydrolysis of (XLI) with aqueous dioxan at 150° gives the triol (XXXV)<sup>67</sup> with inversion at  $C_5$ .

The configurations (XXXIX) and (XLI) assigned to the cholesterol

<sup>70</sup> T. Westphalen and A. Windaus, *Ber.*, 1915, **48**, 1064.

<sup>71</sup> A. Windaus and H. Lüders, *Z. physiol. Chem.*, 1921, **117**, 154.

<sup>72</sup> L. Ruzicka and W. Bosshard, *Helv. Chim. Acta*, 1937, **20**, 244.

<sup>73</sup> R. A. Baxter and F. S. Spring, *J.*, 1943, 613.

<sup>74</sup> Z. Hattori, *J. Pharm. Soc. Japan*, 1940, **60**, 125.

<sup>75</sup> A. Windaus, *Ber.*, 1917, **50**, 133.

<sup>76</sup> M. F. C. Paige, *J.*, 1943, 439.

<sup>77</sup> H. Reich and A. Lardon, *Helv. Chim. Acta*, 1946, **29**, 671.

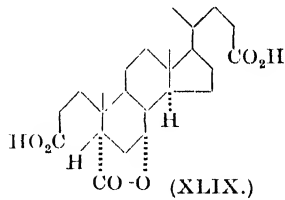
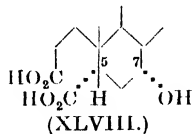
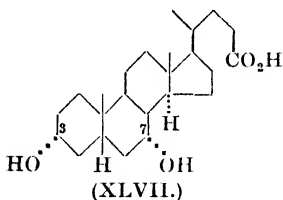
\* Plattner *et al.*<sup>65</sup> actually say “zum Methyl ( $C_{18}$ )”, but this is surely an error for  $C_{10}$ ; when numbers are assigned to the carbon atoms of the angular methyl groups, that attached to  $C_{10}$  is correctly numbered 19 and that attached to  $C_{13}$  is correctly numbered 18.<sup>4, 5, 28</sup>

oxides are in agreement with previous work;<sup>73</sup> use of the very typical optical rotation differences enables corresponding configurations to be assigned to the pairs of oxides derived from stigmaterol,<sup>78</sup> dehydroisoandrosterone,<sup>79</sup> pregn-5-en-3( $\beta$ )-ol-20-one,<sup>80</sup> pregn-5-ene-3( $\beta$ ):21-diol-20-one<sup>81</sup> and pregn-5-en-3( $\beta$ ):20:21-triol.<sup>82</sup> It is doubtful if these observations can be extended to the sterol benzoate oxides of Wallis *et al.*,<sup>83</sup> because they appear to have used molecular compounds of the " $\alpha$ " and " $\beta$ "-forms, and not the true " $\beta$ "-oxides; these authors describe the use of anhydrous hydrogen fluoride at  $-80^\circ$  for a few minutes to cleave oxides.

The course of hydrogenation of steroid 4:5- and 5:6-oxides depends on the conditions used, the configuration of the oxide, and the presence or otherwise of substituents at C<sub>3</sub>. Thus 5:6( $\alpha$ )-oxidocholestane gives cholestane, and 5-hydroxy- and a 6( $\beta$ )-hydroxy-cholestane;<sup>66</sup> 4:5-oxidocholestane gives the same 5-hydroxycholestane and 4( $\beta$ )-hydroxycholestane.<sup>66</sup> Nevertheless therein lies a method permitting the introduction of the 5-hydroxyl group characteristic of many of the cardiac aglycones.

Cholesterol by treatment with iodobenzene dichloride gives 80% of a single dichloride, but esters of cholesterol, stigmaterol, and sitosterol each give two isomeric ester-dichlorides characterised by large differences in m. p. and specific rotation; benzoylation of cholesterol dichloride gives the benzoate-dichloride of lower m. p.<sup>84</sup>

*Position C<sub>7</sub>*.—The 7-hydroxyl group in chenodeoxycholic acid (XLVII) and cholic acid (L) is ( $\alpha$ )-orientated. Hypobromite oxidation of (XLVII) gives a 7-hydroxytricarboxylic acid (XLVIII) which readily yields the lactonic acid<sup>85</sup> (XLIX), also obtained from (L).<sup>86</sup> Chenodeoxycholic acid is a coprostane derivative,<sup>87</sup> hence the C<sub>5</sub>-carboxyl group in (XLIX) is ( $\alpha$ )-orientated, from which it follows that the C<sub>7</sub>-hydroxyl group must also



be ( $\alpha$ )-orientated. This is also true for cholic acid (L) which has been converted into chenodeoxycholic acid (XLVII);<sup>88</sup> an improved conversion

<sup>78</sup> E. Fernholz, *Annalen*, 1934, **508**, 215.

<sup>79</sup> L. Ruzicka and A. C. Muhr, *Helv. Chim. Acta*, 1944, **27**, 503.

<sup>80</sup> M. Ehrenstein and T. O. Stevens, *J. Org. Chem.*, 1941, **6**, 908.

<sup>81</sup> M. Ehrenstein, *ibid.*, p. 626.

<sup>82</sup> *Idem, ibid.*, 1943, **8**, 83.

<sup>83</sup> E. M. Hicks, junr., C. J. Berg, and E. S. Wallis, *J. Biol. Chem.*, 1946, **162**, 633.

<sup>84</sup> C. J. Berg and E. S. Wallis, *ibid.*, p. 683.

<sup>85</sup> A. Windaus, *Z. physiol. Chem.*, 1926, **157**, 181; 1932, **213**, 180.

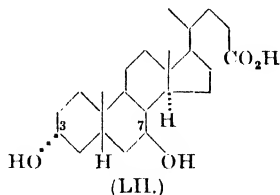
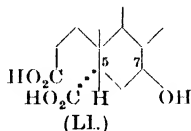
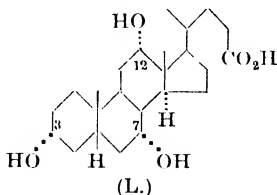
<sup>86</sup> W. Borsche and R. Franck, *Ber.*, 1926, **59**, 1748.

<sup>87</sup> A. Windaus, A. Bohne, and E. Schwarzkopf, *Z. physiol. Chem.*, 1924, **140**, 177.

<sup>88</sup> T. Iwasaki, *ibid.*, 1936, **244**, 181.

(L  $\longrightarrow$  XLVII), which preserved the 7( $\alpha$ )-hydroxyl group intact throughout, has recently been carried out.<sup>21</sup>

Ursodeoxycholic acid is 3( $\alpha$ ):7( $\beta$ )-dihydroxycholanic acid (LII), since by hypobromite oxidation it gives a 7-hydroxy-acid (LI), isomeric with (XLVIII), which does not yield a lactonic acid.<sup>89</sup>



Autoxidation of cholesterol (and other  $\Delta^5$ -steroids) in aqueous colloidal solution gives the epimeric 3( $\beta$ ):7-dihydroxycholestenes<sup>90</sup> (and analogous compounds); the 7" $\beta$ "-compound had previously been prepared,<sup>91</sup> and it now appears that the 7" $\alpha$ "-compound, resulting from 7-ketocholesterol by reduction with aluminium isopropoxide,<sup>92</sup> is a mixture of epimerides containing up to 20% of the 7" $\beta$ "-compound;<sup>93</sup> the same may be expected to be true of one of the epimeric 3( $\alpha$ ):7-dihydroxycholestenes.<sup>94</sup> Although one of the epimeric 3( $\beta$ ):7-dibenzoyloxycholestenes should be subject to steric hindrance at C<sub>7</sub>, both by treatment with cold sodium methoxide give 7-monobenzoates.<sup>93, 95</sup> Differences in reactivity are disclosed in elimination reactions, wherein the 7" $\alpha$ "-compounds readily give cholest-7-ene derivatives, whilst the 7" $\beta$ "-compounds are resistant.<sup>93, 96</sup>

There is thus no direct evidence upon which an assignment of configuration at C<sub>7</sub> in the sterol series can be based;<sup>97</sup> a correlation with the known orientation of the 7-hydroxyl group in the bile acids (XLVII), (L), and (LII) is much to be desired. Indirect evidence based on optical rotatory powers permits, however, a provisional allocation. Wintersteiner and Moore<sup>98</sup> gave the epimeric 3( $\beta$ ):7-dihydroxycholestane with the more positive rotation the " $\alpha$ "-configuration; they unfortunately expressed this arbitrary assignment not by use of the usual trivial index " $\alpha$ ", but by employment of the expression ( $\alpha$ ), in which the parentheses should denote established spatial orientation. It appears that their arbitrary assignment of configuration, and also that of Windaus and Naggatz<sup>94</sup> for the

<sup>89</sup> S. Kawai, *Z. physiol. Chem.*, 1933, **214**, 71.

<sup>90</sup> O. Wintersteiner and S. Bergstrom, *J. Biol. Chem.*, 1941, **137**, 785; 1941, **141**, 597; 1942, **143**, 503.

<sup>91</sup> T. Barr, I. M. Heilbron, E. G. Parry, and F. S. Spring, *J.*, 1936, 1437.

<sup>92</sup> A. Windaus, H. Lettre, and F. Schenk, *Annalen*, 1935, **520**, 98; U.S.P. 2,098,985 (1937).

<sup>93</sup> O. Wintersteiner and W. L. Ruigh, *J. Amer. Chem. Soc.*, 1942, **64**, 2453.

<sup>94</sup> A. Windaus and J. Naggatz, *Annalen*, 1939, **542**, 204.

<sup>95</sup> O. Wintersteiner and W. L. Ruigh, *J. Amer. Chem. Soc.*, 1942, **64**, 1177.

<sup>96</sup> O. Wintersteiner and M. Moore, *ibid.*, 1943, **65**, 1507.

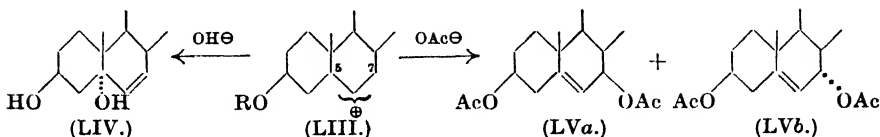
<sup>97</sup> L. Ruzicka, V. Prelog, and E. Tagmann, *Helv. Chim. Acta*, 1944, **27**, 1149.

<sup>98</sup> O. Wintersteiner and M. Moore, *J. Amer. Chem. Soc.*, 1943, **65**, 1503.



7-epimeric 3( $\alpha$ ) : 7-dihydroxycholest-5-enes, and of Reichstein and Fuchs<sup>99</sup> for the 7-epimeric methyl 3( $\beta$ ) : 7-diacetoxyætio-5-*allo*cholanates should be reversed. The molecular rotations of (a) lithocholic, chenodeoxycholic, and ursodeoxycholic acids are +121°, +49°, and +224° and (b) 3( $\beta$ )-hydroxy-, 3( $\beta$ ) : 7“ $\beta$ ”-dihydroxy-, and 3( $\beta$ ) : 7“ $\alpha$ ”-dihydroxycholestane are +89°, and +33°, and +214°. The  $[M]_D$  differences for reversal of configuration at C<sub>7</sub> are (a) +175°; (b) +181°; for the introduction of epimeric 7-hydroxyl groups, the contributions by difference are : (a) -72°, (b) -56°, and (a) +103°, (b) +125°. Clearly, if analogies concerning optical rotatory power can be drawn between the coprostanes and the cholestane series, the indices extant for C<sub>7</sub> in the latter must be reversed. Plattner and Heusser<sup>21</sup> have drawn attention to the discrepancy, whilst such reversal has been accepted by Reichstein and Grand.<sup>100</sup> On this basis, it is not, however, possible to account for the relative ease with which 7( $\beta$ )-epimerides [7( $\beta$ )-substituent/8-H : *cis*] give cholest-7-ene derivatives by elimination, whilst the 7( $\alpha$ )-epimerides [7( $\alpha$ )-substituent/8-H : *trans*] are resistant. In the cholic acid series where the relation, 7( $\alpha$ )-OH/8-H : *trans*, is established, dehydration under very mild conditions to chol-7-ene derivatives has been observed.<sup>100</sup>

The analogous epimeric 3( $\beta$ ) : 7-dihydroxycholest-5-enes<sup>91, 94</sup> and their derivatives fall into two series showing respectively positive and negative specific rotations :<sup>98</sup> owing to the possibility of “vicinal” action,<sup>22</sup> the evidence here is less clear but it seems probable that the epimeride with  $[\alpha]_D + 7^\circ$  is the 3( $\beta$ ) : 7( $\beta$ )-compound and the epimeride with  $[\alpha]_D - 87^\circ$  is the 3( $\beta$ ) : 7( $\alpha$ )-compound. If this allocation should prove correct, the dextro-“ $\alpha$ ”- and lævo-“ $\beta$ ”-cholest-5-enetriols<sup>101</sup> are respectively 3( $\beta$ ) : 4( $\beta$ ) : 7( $\beta$ )- and 3( $\beta$ ) : 4( $\beta$ ) : 7( $\alpha$ )-trihydroxycholest-5-ene. Whilst 3( $\beta$ ) : 7( $\alpha$ )-diacetoxycholest-5-ene and 3( $\beta$ ) : 7( $\beta$ )-diacetoxycholest-5-ene may be subjected to alkaline hydrolysis and the products reacylated using acetic anhydride-pyridine at 20° with complete preservation of individuality,<sup>97</sup> treatment of either epimeride with hot acetic acid gives the same mixture of both epimerides. This appears to result from triad anionotropy; each epimeride singly gives rise to the flattened mesomeric cation (LIII; R = Ac) from which both epimerides (LVa), (LVb) can be derived by recombination with an acetate anion at C<sub>7</sub>. Under other conditions coordination of an anion occurs at C<sub>5</sub>; thus 3( $\beta$ )-acetoxy-7“ $\beta$ ”-bromocholest-5-ene by alkaline hydrolysis is converted *via* (LIII; R = H) into



3( $\beta$ ) : 5-dihydroxycholest-6-ene (LIV), hydrogenated to the saturated diol

<sup>99</sup> T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, 1939, **22**, 1160.

<sup>100</sup> R. Grand and T. Reichstein, *ibid.*, 1945, **28**, 344, 346, footnote 2.

<sup>101</sup> V. A. Petrow and W. W. Starling, *J.*, 1946, 749.

(XL), non-production of the analogous coprost-6-ene compound being in accordance with theoretical expectation (Henbest and Jones, *loc. cit.*, in footnote).\*

*Position C<sub>8</sub> and C<sub>9</sub>.*—The junction of rings B and C is regarded as *trans*-to fit the requirements of X-ray measurements,<sup>3</sup> but Bernal<sup>28</sup> states "this determination has not anything like the certainty of that of the parallel decision in the case of the *cis*- and *trans*-hexahydrochrysenes. In the completely reduced ring-system there is much greater freedom of movement and models can be constructed with a *cis*-central junction nearly as flat as those where the junction is *trans*. But where one ring is aromatic as in œstrone, the more rigid conditions hold and here it is certain that the central junction must be *trans*". He also states that to keep the molecule flat the angular methyl group at C<sub>10</sub> must in general be *trans* to the hydrogen atom at C<sub>9</sub>.

The suggestion of *cis*-B/C ring-fusion has been made in the case of only two natural products. R. Tschesche and K. Bohle<sup>102</sup> converted sarmentogenin into a dehydrolactone also obtained from digoxigenin; they regarded these aglycones as isomeric 3(α) : 11 : 14-trihydroxy-compounds and referred the isomerism to a *cis*-B/C ring-union in sarmentogenin. Digoxigenin is, however, a 3(α) : 12(β) : 14-trihydroxy-compound, oxidised by chromium trioxide to a 14-hydroxy-3 : 12-diketone, whereas sarmentogenin under identical conditions yields an isomeric 14-hydroxy-diketone.<sup>103</sup> It is clear that sarmentogenin must differ from digoxigenin apart from epimerism, but that the assumption of a *cis*-B/C ring-fusion is no longer necessary.

R. E. Marker *et al.*<sup>104</sup> have suggested that a *cis*-B/C linkage is present in their urane-derivatives, but as their several papers exhibit serious inconsistencies, this claim need not at present further be considered.

*Position C<sub>11</sub> and C<sub>12</sub>.*—The extraordinarily unreactive 11-hydroxyl group characteristic of some of the natural cortical steroids was originally assigned the (β)-configuration<sup>105</sup> because that configuration, in contrast to the (α)-orientation, is greatly hindered by *both* the angular methyl groups at C<sub>10</sub> and C<sub>13</sub>.

Subsequently, epimeric 11 : 12-oxides (A and B) were obtained; (A) from Δ<sup>11</sup>-compounds (LVI) by filtration of the bromohydrins derived therefrom over aluminium oxide,<sup>106, 107</sup> and (B) directly from (LVI) by use of

<sup>102</sup> *Ber.*, 1936, **69**, 793, 2497.

<sup>103</sup> A. Katz and T. Reichstein, *Pharm. Acta Helv.*, 1944, **19**, 231.

<sup>104</sup> *J. Amer. Chem. Soc.*, 1938, **60**, 210, 1061, 1559, 1561.

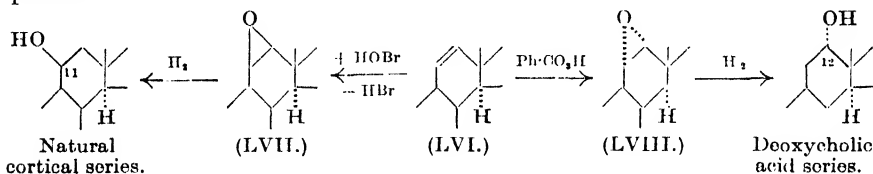
<sup>105</sup> T. Reichstein and C. W. Shoppee, "Vitamins and Hormones", New York, 1943, p. 345.

<sup>106</sup> H. Reich and T. Reichstein, *Helv. Chim. Acta*, 1943, **26**, 562.

<sup>107</sup> G. H. Ott and T. Reichstein, *ibid.*, p. 1799.

\* Since this report was written, the compounds formerly regarded as 3(β) : 5-dihydroxycholest-6-ene<sup>90</sup> and 3(β) : 5-dihydroxycoprost-6-ene (V. Prelog, L. Ruzicka, and P. Stein, *Helv. Chim. Acta*, 1943, **26**, 2222) have been shown to be the 7-methyl and 7-ethyl ethers respectively of 3(β) : 7"β"-dihydroxycholest-5-ene (LVa or LVb, with OH for OAc) (H. B. Henbest and E. R. H. Jones, *Nature*, 1946, **158**, 950).

perbenzoic acid.<sup>108, 109, 110</sup> The oxides (A) and (B) by hydrogenation gave respectively 11- and 12-hydroxy-compounds, the latter being identical with derivatives of deoxycholic acid. At that time (1943—46), deoxycholic acid was regarded as a 12( $\beta$ )-hydroxy-compound, and the oxides (B) were therefore regarded as having the 11( $\beta$ ) : 12( $\beta$ )-configuration, whilst the oxides (A) were assigned the 11( $\alpha$ ) : 12( $\alpha$ )-configuration. It has now been proved (*vide infra*) that the 12-hydroxyl group in deoxycholic acid is ( $\alpha$ )-orientated; hence the configurations formerly assigned to the epimeric oxides (A) and (B) must be inverted, and (A) and (B) are respectively (LVII) and (LVIII). The 11-hydroxy-compounds derived from (LVII) are identical with those obtained by reduction of the 11-keto-compounds<sup>111</sup> synthesised by the method of H. Reich and T. Reichstein;<sup>106, 112</sup> from one of these corticosterone has been synthesised,<sup>113</sup> so that corticosterone and the natural cortical substances with a hydroxyl group at C<sub>11</sub> (except substances C and R\* which have not yet been shown to belong to the same stereochemical series in respect of C<sub>11</sub> as their congeners) have the ( $\beta$ )-configuration at that position.



The 11( $\alpha$ )-epimeride of corticosterone was obtained by T. Reichstein and C. W. Shoppee<sup>114</sup> under conditions which imply its resistance to dehydration; recently 11( $\alpha$ )-hydroxy-steroids have been synthesised, and differ from the 11( $\beta$ )-epimerides, which are readily dehydrated<sup>115</sup> but acetylated with extreme difficulty,<sup>116</sup> by being resistant to dehydration and acetylated with relative ease. The 11( $\alpha$ ) : 12( $\alpha$ )-oxide (LIX) undergoes acetolysis to give (LX; R<sup>1</sup> = R<sup>2</sup> = H), the 11( $\beta$ )-hydroxyl group of which resists hydrolysis;<sup>109</sup> an improved preparation<sup>117</sup> leads to (LX; R<sup>1</sup> = Ac, R<sup>2</sup> = Me), oxidised by chromium trioxide to (LXI). The 11( $\beta$ )-acetoxyl group of (LXI) undergoes hydrolysis on account of activation by the 12-keto-

<sup>108</sup> H. Press and T. Reichstein, *Helv. Chim. Acta*, 1942, **25**, 821.

<sup>109</sup> T. F. Gallagher and W. P. Long, *J. Biol. Chem.*, 1946, **162**, 495.

<sup>110</sup> B. F. McKenzie, W. F. McGuckin, F. Warren, E. C. Kendall, *ibid.*, p. 555.

<sup>111</sup> A. Lardon and T. Reichstein, *Helv. Chim. Acta*, 1943, **26**, 586.

<sup>112</sup> For an excellent summary of this method see F. S. Spring, *Ann. Reports*, 1943, **40**, 151.

<sup>113</sup> J. von Euw, A. Lardon, and T. Reichstein, *Helv. Chim. Acta*, 1944, **27**, 1287.

<sup>114</sup> *Ibid.*, 1943, **26**, 586.

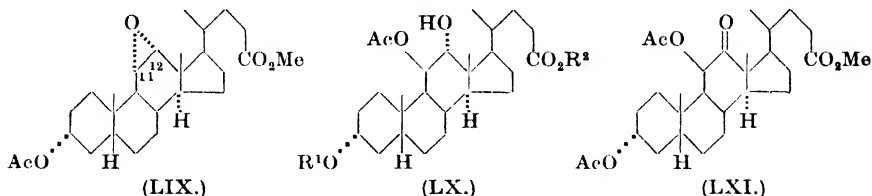
<sup>115</sup> C. W. Shoppee, *ibid.*, 1940, **23**, 740.

<sup>116</sup> M. Steiger and T. Reichstein, *ibid.*, 1937, **20**, 817.

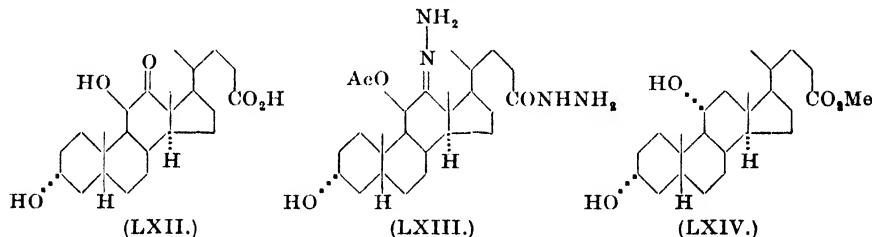
<sup>117</sup> T. F. Gallagher and W. P. Long, *J. Biol. Chem.*, 1946, **162**, 511, 521.

\* Shortly after this report was written, the hydroxyl group at C<sub>11</sub> in substance R was shown to be ( $\beta$ )-orientated (J. von Euw and T. Reichstein, *Helv. Chim. Acta*, 1947, **30**, 205).

group <sup>118</sup> to give the acid (LXII). Treatment of (LX ; R<sup>1</sup> = Ac, R<sup>2</sup> = Me) with hydrazine gives the hydrazido-hydrazone (LXIII) [the 11(β)-acetoxy]



group resisting hydrolysis], Wolff-Kishner reduction of which proceeds with inversion of configuration at C<sub>11</sub> to give, after esterification, methyl 3(α):11(α)-dihydroxycholanate (LXIV) accompanied by methyl lithocholate, methyl lithochol-11-enate, and other products. The 11(α)-hydroxyl group of (LXIV) resists dehydration with hydrochloric-acetic acid, but is readily acetylated and the acetate readily hydrolysed. Wieland-Barbier



degradation of (LXIV) leads to 3(α):11(α)-dihydroxyætiucholanic acid (LXV),<sup>119</sup> also obtained from ætiodeoxycholic acid.<sup>120</sup>

Addition of hydrobromic acid to the oxide <sup>109</sup> (LIX) similarly gives the 11(β)-bromohydrin (XLVI), oxidised by chromium trioxide to the 11(β)-bromo-12-keto-ester (LXVII), which is identical with that one of the pair of epimerides previously obtained as the minor product by bromination of methyl 3(α)-acetoxy-12-ketocholanate<sup>121</sup> (LXVIII). The 11(β)-position being extremely hindered, bromination of (LXVIII) yields little of the 11(β)-bromide (LXVII) and much of the 11(α)-bromide (LXIX); the reactivity of these bromides with pyridine and collidine agrees with the configurations assigned.<sup>109, 121, 122</sup>

Since alkaline hydrolysis of the bromides (LXVII) and (LXIX) should proceed by mechanism *S<sub>N</sub>2* and so be subject to steric hindrance, replacement should occur in both with inversion of configuration<sup>8</sup> but at widely different rates [11(β) ≫ 11(α)]. This is so, since the 11(β)-bromide (LXVII) with *N*/2-aqueous-alcoholic potassium hydroxide at 20° rapidly undergoes inversion to give the 3(α):11(α)-dihydroxy-12-keto-acid (LXX), whilst

<sup>118</sup> H. B. Watson, *Ann. Reports*, 1938, **35**, 246.

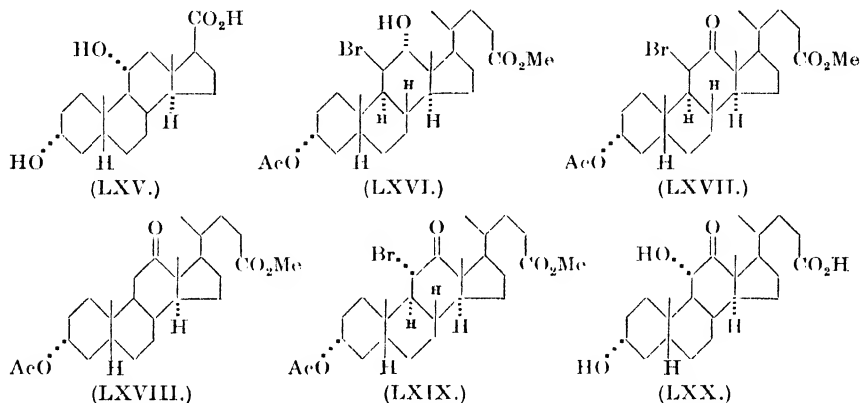
<sup>119</sup> W. P. Long, C. W. Marshall, and T. F. Gallagher, *J. Biol. Chem.*, 1946, **165**, 197.

<sup>120</sup> T. F. Gallagher, *ibid.*, p. 211.

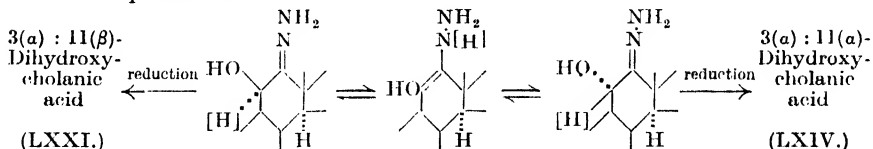
<sup>121</sup> E. Seebeck and T. Reichstein, *Helv. Chim. Acta*, 1943, **26**, 536.

<sup>122</sup> H. B. Alther and T. Reichstein, *ibid.*, p. 492.

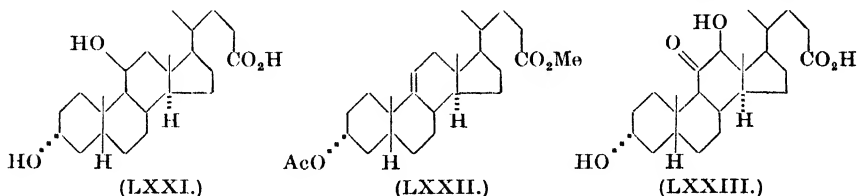
under the same conditions the 11( $\alpha$ )-bromide (LXIX) slowly undergoes inversion to yield the 3( $\alpha$ ):11( $\beta$ )-dihydroxy-12-keto-acid (LXII);<sup>123</sup> whereas (LXX) readily gives a 3( $\alpha$ ):11( $\alpha$ )-diacetate, (LXII) forms only a 3( $\alpha$ )-monoacetate.



Wolff-Kishner reduction of the 11( $\alpha$ )-hydroxy-acid (LXX) gives the same products<sup>124</sup> as are obtained from the 11( $\beta$ )-hydroxy-acid (LXII), in particular the 3( $\alpha$ ):11( $\alpha$ )-dihydroxy-acid (LXIV). To account for inversion of configuration at C<sub>11</sub> in the reduction (LXII  $\rightarrow$  LXIV), it is suggested that the equilibrium:



is established in the presence of ethoxide ions at 200°. It is shown that the 3( $\alpha$ ):11( $\alpha$ )-dihydroxycholanic acid (LXIV) is stable under these conditions, and it is suggested that the apparent non-production of the epimeric 3( $\alpha$ ):11( $\beta$ )-acid is due to elimination of the 11( $\beta$ )-hydroxyl group by dehydration. The 3( $\alpha$ ):11( $\beta$ )-epimeride (LXXI) of (LXIV) is known,<sup>107, 111</sup> and is dehydrated as the 3-monoacetate methyl ester by



thionyl chloride-pyridine at 20° or phosphorus oxychloride-pyridine at 20° to methyl 3( $\alpha$ )-acetoxychol-9(11)-enate (LXXII). The ætioanalogue of

<sup>123</sup> T. F. Gallagher and W. P. Long, *J. Biol. Chem.*, 1946, **162**, 521.

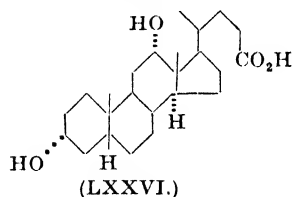
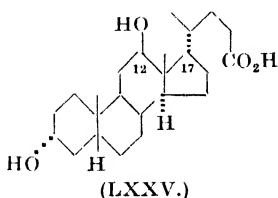
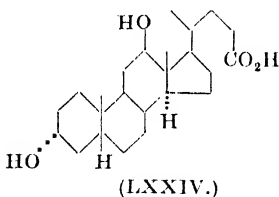
<sup>124</sup> T. F. Gallagher and V. P. Hollander, *ibid.*, p. 533.

(LXX) <sup>120</sup> by Wolff-Kishner reduction similarly gives only the 3( $\alpha$ ):11( $\alpha$ )-dihydroxy $\alpha$ tiocolanic acid (LXV) together with 3( $\alpha$ ):11:12-trihydroxy- $\alpha$ tiocolanic acids.<sup>119</sup>

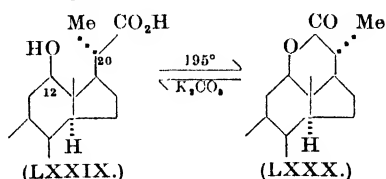
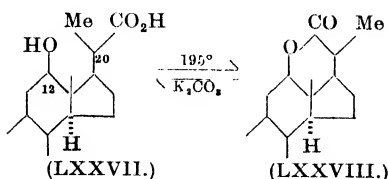
Neither (LXII) nor (LXX) is identical with the acid thought by Marker and Lawson <sup>125</sup> to be a 3( $\alpha$ ):11-dihydroxy-12-ketocolanic acid; this acid has been found <sup>126, 127, 128, 129</sup> to be 3( $\alpha$ ):12( $\beta$ )-dihydroxy-11-ketocolanic acid (LXXIII). It is obtained from both the epimeric bromides (LXVII) and (LXIX) by vigorous treatment with potassium hydroxide. As by-products of these investigations several 3( $\alpha$ ):11:12-trihydroxycolanic acids are obtained; all four acids epimeric at C<sub>11</sub> and C<sub>12</sub> are now known and configurations have been assigned to each.<sup>126, 127, 128</sup> The  $\alpha$ tiocolanic analogue of the acid (LXXIII) has been obtained from 3( $\alpha$ ):11( $\alpha$ )-dihydroxy-12-ketocolanic acid ( $\alpha$ tiocolanic acid (LXX)) by treatment at 20° with 2N-potassium hydroxide.<sup>120</sup>

Deoxycholic acid, previously considered on the basis of Giacomello's <sup>2</sup> X-ray crystallographic measurements to be 3( $\alpha$ ):12( $\beta$ )-dihydroxycolanic acid (LXXIV), and during the period 1943—46 regarded as 3( $\alpha$ ):12( $\beta$ )-dihydroxy-17-*isocolanic* acid (LXXV), has now been proved to be 3( $\alpha$ ):12( $\alpha$ )-dihydroxycolanic acid (LXXVI); the other known bile acids possess corresponding constitutions.

Reichstein *et al.*<sup>16, 130, 131</sup> observed that 12-keto-steroids by hydrogenation with Raney nickel in alkaline solution give preferentially 12-*epi*-hydroxy-compounds, *i.e.*, the 12( $\beta$ )-hydroxy-epimerides. By use of this observation M. Sorkin and T. Reichstein <sup>132</sup> synthesised 12-*epi*-20-*n*-bisor-



deoxycholic acid (LXXVII) and 12-*epi*-20-*isobisordeoxycholic* acid (LXXIX) and found them under drastic conditions to give  $\delta$ -lactones (LXXVIII) and (LXXX.)



<sup>125</sup> R. E. Marker and E. J. Lawson, *J. Amer. Chem. Soc.*, 1938, **60**, 1334.

<sup>126</sup> T. F. Gallagher, *J. Biol. Chem.*, 1946, **162**, 539.

<sup>127</sup> O. Wintersteiner, M. Moore, and K. Reinhardt, *ibid.*, p. 707.

<sup>128</sup> E. Berner and T. Reichstein, *Helv. Chim. Acta*, 1946, **29**, 1374.

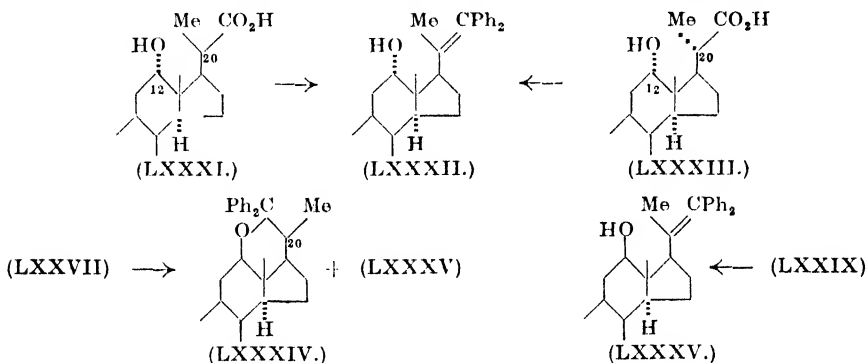
<sup>129</sup> T. F. Gallagher and E. Borgstrom, *J. Biol. Chem.*, 1946, **164**, 791.

<sup>130</sup> M. Sorkin and T. Reichstein, *Helv. Chim. Acta*, 1943, **26**, 2097.

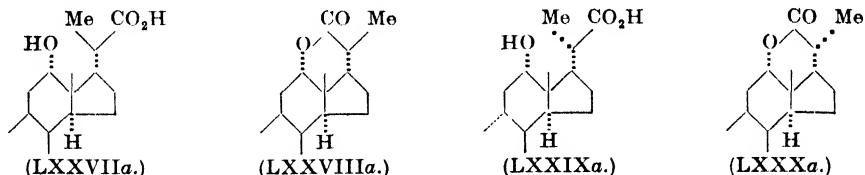
<sup>131</sup> W. Wenner and T. Reichstein, *ibid.*, 1944, **27**, 965.

<sup>132</sup> *Ibid.*, p. 1631.

and (LXXX), which were quantitatively reconverted into the parent acids by hydrolysis with potassium carbonate. The  $C_{12}$ -hydroxyl group and the  $C_{17}$ -side chain must therefore lie on the same side of the general plane of the ring-system in these 12-*epi*-acids and on opposite sides in natural deoxycholic acid. This conclusion was supported by an examination<sup>133</sup> of the behaviour of the methyl esters of the 12-*epi*-acids (LXXVII), (LXXIX), 20-*n*-bisordeoxycholic acid (LXXXI), and 20-*isobis*ordeoxycholic acid (LXXXIII) with phenylmagnesium bromide. Dehydration of the diphenylcarbinols obtained from the esters of the 12-*n*-acids (LXXXI) and (LXXXIII), which results in disappearance of the centre of asymmetry at  $C_{20}$ , gave the same diphenylethylene (LXXXII) but no trace of a cyclic oxide. Similar dehydration of the diphenylcarbinols resulting from the 12-*epi*-acids (LXXVII) and (LXXIX) gave the diphenylethylene (LXXXV) together, in the case of (LXXVII), with the cyclic oxide (LXXXIV).



Giacomello's formulation (LXXIV) of deoxycholic acid is therefore wrong, but because Sorkin and Reichstein<sup>132</sup> regarded his *X*-ray evidence as stronger in respect of the  $C_{12}$ -orientation, they preferred to formulate the compounds (LXXVII—LXXX) as (LXXVIIa—LXXXa).



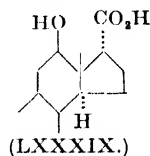
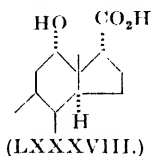
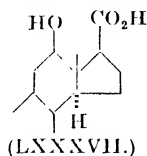
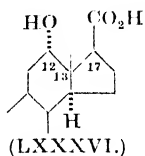
M. Sorkin and T. Reichstein<sup>134</sup> have examined the capacity for  $\gamma$ -lactone formation of the four possible ætiodeoxycholic acids (LXXXVI, LXXXVII, LXXXVIII, and LXXXIX) differing in configuration at  $C_{12}$  and  $C_{17}$ . Neither ætiodeoxycholic acid<sup>135</sup> (LXXXVI) nor 12-*epi*ætiodeoxycholic

<sup>133</sup> M. Sorkin and T. Reichstein, *Helv. Chim. Acta*, 1945, **28**, 875.

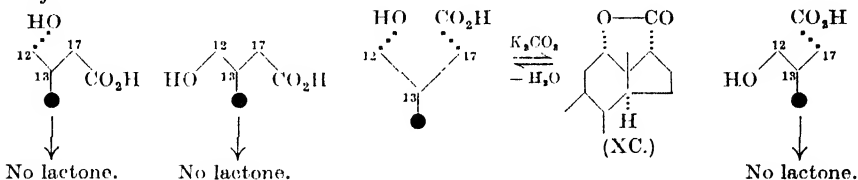
<sup>134</sup> *Ibid.*, 1946, **29**, 1218.

<sup>135</sup> W. M. Hoehn and H. L. Mason, *J. Amer. Chem. Soc.*, 1938, **124**, 459; E. von Arx and T. Reichstein, *Helv. Chim. Acta*, 1940, **23**, 747.

acid <sup>131</sup> (LXXXVII) yields a  $\gamma$ -lactone under drastic conditions. By epimerisation with sodium methoxide,<sup>136</sup> these two acids have been converted into 17-*iso*- $\alpha$ -tiodeoxycholic acid (LXXXVIII) and 12-*epi*-17-*iso*- $\alpha$ -tiodeoxycholic acid (LXXXIX) respectively. The latter cannot be lactonised, but (LXXXVIII) passes into the  $\gamma$ -lactone (XC) with extreme ease. The lactone (XC) is also produced quantitatively by sublimation in a high vacuum of the methyl ester of the acid (LXXXVIII), and is reconverted into the parent acid by hydrolysis with potassium carbonate.



*Projection*



It might be thought that, by analogy with the higher homologues (LXXVII) and (LXXIX), the acid (LXXXVII) should lactonise under sufficiently drastic conditions; apparently, the  $C_{13}$ -angular methyl group here constitutes an additional and unsurmountable obstacle.<sup>137</sup>

The new formula (LXXXVI) for deoxycholic acid has recently been proposed by Long and Gallagher<sup>109</sup> on entirely different grounds and by reference to the centre of asymmetry at  $C_9$ , the stereochemical relationship of which to the angular methyl group at  $C_{10}$  is rather well established (see p. 217); their experimental results demonstrate the intermediate formation of 11-halogeno-12-hydroxy-compounds in the catalytic reduction in presence of halogen acid (HX) of the oxide (LIX; cf. LVIII) to derivatives of deoxycholic acid.<sup>110</sup> The structure of this intermediate (when  $HX = HBr$ ) is known<sup>109, 117, 121</sup> to be (LXVI), on account of its conversion by oxidation into the 11( $\beta$ )-bromo-ketone [(LXVII);  $C_{11}-Br/C_9-H$ , *trans*] which by treatment with hot pyridine for 3 hours gives an excellent yield of methyl 3( $\alpha$ )-acetoxy-12-ketochol-9(11)-enate, whereas the 11( $\alpha$ )-epimeride (LXIX;  $C_{11}-Br/C_9-H$ : *cis*) is recovered unchanged under these conditions. The  $C_{12}$ -hydroxyl group in deoxycholic acid must accordingly have the same ( $\alpha$ )-orientation as the  $C_{12}$ -hydroxyl group in (LXVI). The rates of alkaline hydrolysis ( $S_N2$ ) of 12-acetoxy groups [ $12(\beta) > 12(\alpha)$ ] found by Koechlin and Reichstein<sup>16</sup> are discordant, since a  $12(\beta)$ -acetoxy group should be more hindered by the  $C_{13}$ -angular methyl group than a  $12(\alpha)$ -acetoxy group

<sup>136</sup> M. Sorkin and T. Reichstein, *Helv. Chim. Acta*, 1946, **29**, 1209.

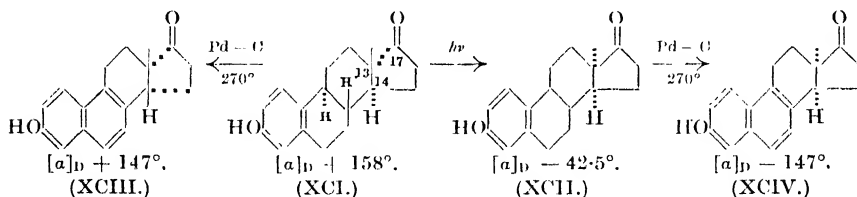
<sup>137</sup> C. W. Shoppee, *Chem. and Ind.*, 1947, 109.



(cf. p. 204); but, as the authors point out, it is possible that the observed steric hindrance is due to the long side-chain at C<sub>17</sub> rather than the angular methyl group at C<sub>13</sub>.

*Positions C<sub>13-17</sub>.*—There is as yet no X-ray evidence on the junction between rings C and D, but chemical evidence indicates that ring-fusion is *trans* in the bile-acids<sup>138</sup> and the sterols.<sup>139</sup> Natural steroids generally have been regarded as having a *trans*-C/D union, and, the C<sub>13</sub>-methyl group being by convention ( $\beta$ )-orientated, (+)- $\alpha$ -estrone has commonly been represented by (XCI).

Irradiation of (+)- $\alpha$ -estrone (XCI) with monochromatic ultra-violet light of wave-length 313 m $\mu$  leads by an irreversible one-quantum process with inversion at C<sub>13</sub> to lumicestrone (XCII),<sup>140</sup> whilst androsterone gives lumiandrosterone.<sup>141</sup> Only configuration at C<sub>13</sub> is altered for, if inversion occurred also C<sub>14</sub>, the product would be the as yet unknown (–)- $\alpha$ -estrone,



[ $\alpha$ ]<sub>D</sub> –158°. The 17-keto-group in (XCII) is very unreactive, but Wolff-Kishner reduction gives 17-deoxylumicestrone, which cannot be obtained by irradiation of 17-deoxy $\alpha$ -estrone. Dehydrogenation of (+)- $\alpha$ -estrone (XCI) gives, surprisingly, not (+)-equilenin, but (+)-*isoequilenin*<sup>142</sup> with inversion of configuration at C<sub>14</sub>, whilst the parallel experiment with lumicestrone (XCII) gives the enantiomorphous (–)-*isoequilenin* without inversion at C<sub>14</sub>. Inversion must be assumed to occur in *one* of these dehydrogenations, even if *isoequilenin* has, not as previously supposed a *cis*-C/D-union (XCIH, XCIV), but the *trans*-C/D-union hitherto attributed to equilenin (XCVII) by analogy with  $\alpha$ -estrone (XCI).

The stereochemical problem of the structure of equilenin and *isoequilenin* also appears in connexion with the bisdehydromarrrianolic and bisdehydrodoisynolic acids.<sup>143</sup> (+)- $\alpha$ -Estrone (XCI) gives (+)-marrrianolic acid (XCV), convertible into the highly  $\alpha$ -estrogenic (+)-doisynolic acid (XCVI); (+)-equilenin (XCVII) gives a (+)-“ $\beta$ ”-bisdehydromarrrianolic acid (XCVIII),

<sup>138</sup> H. Wieland and E. Dane, *Z. physiol. Chem.*, 1933, **216**, 91.

<sup>139</sup> K. Dimroth and H. Jonsson, *Ber.*, 1941, **74**, 520.

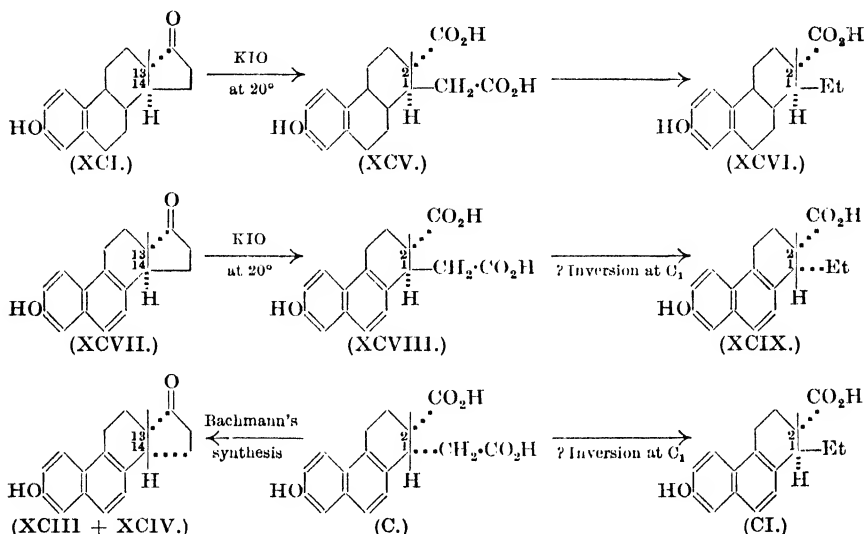
<sup>140</sup> A. Butenandt, A. Wolff, and P. Karlson, *ibid.*, p. 1308; A. Butenandt, W. Friedrich, and L. Poschmann, *ibid.*, 1942, **75**, 1931; A. Butenandt and L. Poschmann, *ibid.*, 1944, **77**, 392.

<sup>141</sup> A. Butenandt and L. Poschmann, *ibid.*, p. 394.

<sup>142</sup> W. E. Bachmann, W. Cole, and A. L. Wilds, *J. Amer. Chem. Soc.*, 1940, **62**, 824.

<sup>143</sup> K. Miescher *et al.*, *Helv. Chim. Acta*, 1944, **27**, 1727; 1945, **28**, 156, 991, 1326, 1506; 1946, **29**, 586, 859, 1071, 1231, 1889, 1894. In the earlier papers, the prefixes “*n*” and “*iso*” were used but have been superseded by “ $\alpha$ ” and “ $\beta$ ” respectively.

but this is converted by the same reaction sequence into the almost non-œstrogenic (+)-“ $\beta$ ”-bisdehydrodoisynolic acid (XCIX), and similarly the racemic “ $\alpha$ ”-bisdehydromarrianolic acid (C), from which Bachmann *et al.*<sup>142</sup> synthesised racemic *isoequilenin* (XCIII and XCIV), yields the intensely œstrogenic racemic “ $\alpha$ ”-bisdehydrodoisynolic acid (CI).



Retention of the existing formulæ for the doisynolic acids (XCVI, XCIX, and CI), whereby the biologically active acids (XCVI and CI) have the same configuration at  $C_1$  and  $C_2$ , requires (a) inversion at  $C_1$  in the last two conversions but not in the first conversion, or (b) that the formulæ (XCVII) and (XCIII + XCIV) previously attributed to equilenin and *isoequilenin* respectively must be interchanged, corresponding configurational adjustments being made to formulæ (XCVIII) and (C).

From the proof<sup>134</sup> that the  $C_{17}$ -side chain in the bile-acids is ( $\beta$ )-orientated, together with the evidence adduced by C. H. Carlisle and D. Crowfoot<sup>144</sup> that this configuration is also present in cholesteryl iodide, it follows that the  $C_{17}$ -side chain in the sterols, the steroid sapogenins,<sup>145</sup> progesterone, corticosterone and its congeners, and the *Digitalis* heart poisons is ( $\beta$ )-orientated. For the *Digitalis* compounds (CII), it is necessary therefore to assume either that the  $C_{14}$ -hydroxyl group is also ( $\beta$ )-orientated, *i.e.*, that rings C and D are *cis*-fused, to permit the formation of *isoglycosides* and *isoaglycones* (CII  $\longrightarrow$  CIII), or that the previously suggested mechanism<sup>146</sup> of the irreversible formation of such *isolactones* is incorrect. The second alternative seems improbable in view of the formation from gitoxigenin<sup>147</sup>

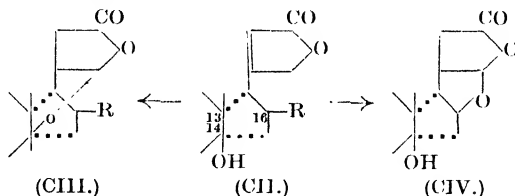
<sup>144</sup> *Proc. Roy. Soc., A*, **184**, 64.

<sup>145</sup> R. E. Marker and D. L. Turner, *J. Amer. Chem. Soc.*, **1941**, **63**, 767.

<sup>146</sup> C. W. Shoppee, *Ann. Rev. Biochem.*, **1942**, **11**, 125 *et seq.*

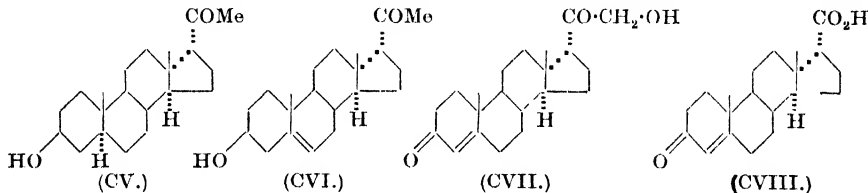
<sup>147</sup> K. Meyer, *Helv. Chim. Acta*, **1946**, **29**, 718.

(CII; R = OH), which has been proved to possess a 16-hydroxyl group,<sup>148</sup> of *two* isomeric isolactones (CIII; R = OH) and (CIV). Ruzicka *et al.*<sup>149</sup> take the first view: "Die natürlichen Aglykone, für welche normale Lage der Butenolid-Gruppe und *cis*-Stellung des Hydroxyls zur letzteren auf Grund ihrer Umsetzungen abgeleitet werden kann, entsprechen demnach dem Typus der Verbindung (CII)".

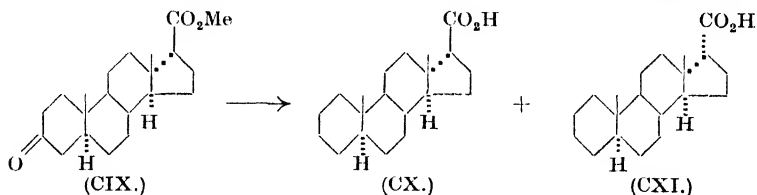


Recently, numerous steroids containing a *cis*-C/D-ring union have been synthesised, but before considering them it is necessary to deal with the synthetic production of 17( $\alpha$ )-substituted steroids termed 17-*iso*-compounds.\*

The 17-*iso*-ketones (CV), (CVI), and 17-*isopro*gesterone were obtained by Butenandt *et al.*,<sup>150, 151</sup> whilst 17-*isodeoxycorticosterone* (CVII) was synthesised and degraded to the 17-*iso*-acid (CVIII) by C. W. Shoppee,<sup>152</sup> who found that whilst (CVII), like (CV) and (CVI), was readily and almost completely epimerised at C<sub>17</sub> by hydrogen ions to give the 17-*n*-ketone, neither (CVIII) nor its methyl ester could be epimerised at C<sub>17</sub> by hydroxyl



ions. J. von Euw and T. Reichstein,<sup>153</sup> however, observed that Wolff-Kishner reduction of methyl 3-keto $\alpha$ tio-5-*allo*cholanate (CIX) gave ordinary



<sup>148</sup> K. Meyer, *Helv. Chim. Acta*, 1946, **29**, 1580.

<sup>149</sup> P. A. Plattner, L. Ruzicka, H. Heusser, J. Pataki, and K. Meier, *ibid.*, p. 945.

<sup>150</sup> A. Butenandt and L. Mamoli, *Ber.*, 1935, **68**, 1847.

<sup>151</sup> A. Butenandt and G. Fleischer, *ibid.*, 1937, **70**, 96; A. Butenandt, J. Schmidt-Thomé, and H. Paul, *ibid.*, 1939, **72**, 1112.

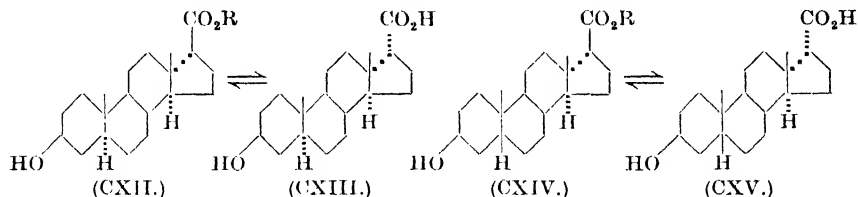
<sup>152</sup> *Helv. Chim. Acta*, 1940, **23**, 925.

<sup>153</sup> *Ibid.*, 1944, **27**, 1851.

\* When the 17-substituent is not separately specified in the name of a compound, e.g., the 17-carboxyl group in  $\alpha$ tio-5-*allo*cholanate (CX), the usual suffix, ( $\alpha$ ) or ( $\beta$ ), cannot readily be used and the prefixes *n*- or *iso*- are convenient.

ætio-5-*allo*cholanolic acid (CX) accompanied by a little 17-*iso*ætio-5-*allo*-cholanolic acid (CXI).

Sorkin and Reichstein<sup>136</sup> showed that treatment of the methyl esters (CXII; R = Me) and (CXIV; R = Me) with alkoxide ions in boiling alcoholic solution gives equilibrium mixtures of the 17-epimeric acids—(CXII; R = H), (CXIII); and (CXIV; R = H), (CXV)—respectively, in which the 17-*n*-acids predominate. The acid (CXI) has recently also been obtained from the methyl ester of (CX) by treatment at 200° with ethoxide ions under pressure.<sup>154</sup>



Steroids containing a *cis*-C/D-ring union have been synthesised as follows. Since hydrogenation of the difficultly accessible 14 : 15-oxide of methyl 3(α) : 12(α)-diacetoxychol-14(15)-enate gave ambiguous results,<sup>155</sup> methyl 3(β)-acetoxyætio-5-*allo*chola-14 : 16-dienate (CXVI) was prepared<sup>156</sup> and found to react with only 1 mol. of perbenzoic acid to give the 14(β) : 15(β)-oxide-ester (CXVII). Complete hydrogenation<sup>149, 157</sup> of (CXVII) gives a mixture of four substances; the 14(α) : 17(β)-ester (CXIX), the 14(β)-hydroxy-17(β)-ester (CXX), the 14(β)-hydroxy-17(α)-ester (CXXI), and the 14(β) : 17(α)-ester (CXXII). The ester (CXIX) is, of course, ordinary methyl 3(β)-acetoxyætio-5-*allo*cholanate; the ester (CXXII; C/D-*cis*) is different from the methyl ester of the acid (CXIII; C/D-*trans*) and has been converted by elimination of the 3(β)-hydroxyl group by three separate methods into methyl 17-*iso*ætio-5 : 14-*diallo*cholanate \* (CXXIV; C/D-*cis*), which is different from the methyl ester of the acid (CXI; C/D-*trans*), a non-identity which can only arise from the stereochemical arrangement at C<sub>14</sub>. The esters (CXX) and (CXXI) are the first 14-hydroxy-steroids to be synthesised, and their preparation opens the way to the synthesis of the aglycones of the *Digitalis* group. They are dehydrated quantitatively, by *trans*-elimination of the 14(β)-hydroxyl group with the appropriate C<sub>15</sub>-hydrogen atom, to the 17-epimeric Δ<sup>14(15)</sup>-esters (CXXVI) and (CXXVII) respectively; these, on elimination of the centre of asymmetry at C<sub>17</sub> by introduction of a C<sub>16</sub>:17-double bond by treatment with *N*-bromosuccinimide,<sup>158</sup>

<sup>154</sup> H. Heusser, K. Meier, and L. Ruzicka, *Helv. Chim. Acta*, 1946, **29**, 1250.

<sup>155</sup> P. A. Plattner, L. Ruzicka, and H. Holtermann, *ibid.*, 1945, **28**, 1660.

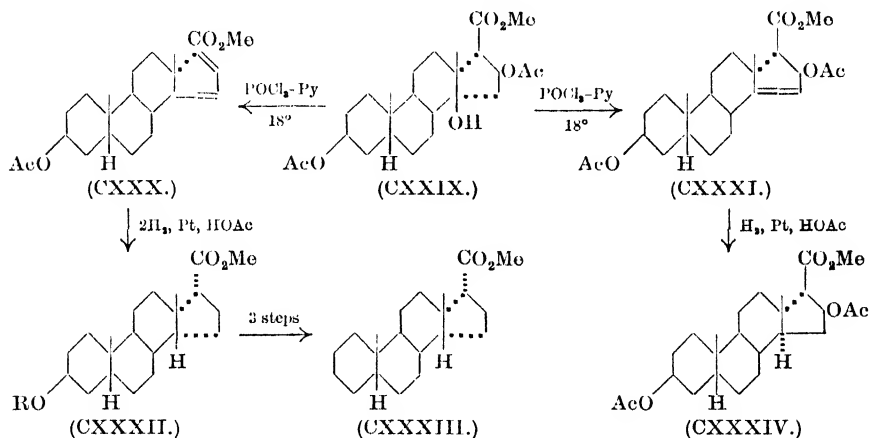
<sup>156</sup> L. Ruzicka, P. A. Plattner, H. Heusser, and J. Pataki, *ibid.*, 1946, **29**, 936.

<sup>157</sup> P. A. Plattner, L. Ruzicka, H. Heusser, and K. Meier, *ibid.*, p. 2023.

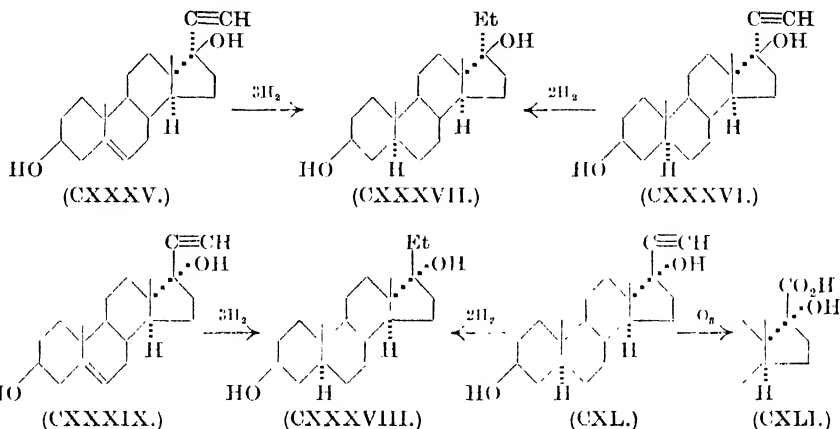
<sup>158</sup> L. Ruzicka, P. A. Plattner, and H. Heusser, *ibid.*, pp. 473, 727.

\* Ruzicka *et al.*<sup>149</sup> use the term 14-*allo*- to describe compounds with a *cis*-C/D ring union; this may lead to confusion because the term 5-*allo*- has long been employed to indicate a *trans*-A/B ring fusion.





the hydroxyl group at C<sub>17</sub> with that to which belongs the 3(β):17"α"-dihydroxy-5-*allopregnane* (CXXXVII), obtained by treatment of *iso*-androsterone with ethylmagnesium bromide<sup>161</sup> or by hydrogenation of the 17-ethynyl compounds (CXXXV) and (CXXXVI) which constitute >99% of the products formed by addition of acetylene to 3(β)-hydroxy-androst-5-en-17-one and *iso*androsterone respectively.<sup>162</sup>



The isolation<sup>162</sup> of the traces (<1%) formed of the 17"β"-hydroxy-epimerides (CXXXIX), (CXL) of (CXXXV), and (CXXXVI), and the degradation<sup>162</sup> of (CXL) to the acid (CXLI) obtained from substance P,<sup>163</sup> similarly only demonstrate that the natural cortical substances belong to the stereochemical series previously, arbitrarily, and incorrectly labelled 17"β"-hydroxy.\*

Ruzicka, Furter, and Goldberg<sup>15</sup> examined the rates of alkaline hydrolysis (S<sub>N</sub>2) of the 17-epimeric acetates of *cis*- and *trans*-dihydrotestosterone and of *cis*- and *trans*-testosterone, and assigned the 17(α)-hydroxy-configuration (C<sub>13</sub>-Me/C<sub>17</sub>-OH: *trans*) to more readily hydrolysed compounds. There appears, however, to be as yet no proof that such indices correspond with the revised indices of the natural and synthetic 17-hydroxy-compounds referred to above, although this seems very probable.

Four oestriols epimeric at C<sub>15</sub> and C<sub>16</sub> should exist (CXLII—CXLV). Natural oestriol should be either (CXLII) or (CXLV) because its 3-methyl ether does not form an acetonide,<sup>165</sup> and, contrary to the view of N. K.

<sup>161</sup> L. Ruzicka, M. W. Goldberg, and H. R. Rosenberg, *Helv. Chim. Acta*, 1935, **18**, 1487; 1936, **19**, 357.

<sup>162</sup> T. Reichstein and C. Meystre, *ibid.*, 1939, **22**, 728.

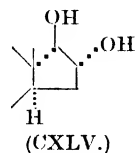
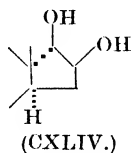
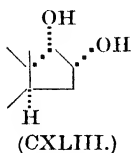
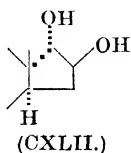
<sup>163</sup> T. Reichstein and K. Gätzi, *ibid.*, 1938, **21**, 1185.

<sup>164</sup> D. A. Prins and T. Reichstein, *ibid.*, 1940, **23**, 1490.

<sup>165</sup> M. N. Huffmann and M. H. Lott, *J. Biol. Chem.*, 1946, **164**, 785.

\* Unfortunately, (α) and (β), instead of trivial indices, "α" and "β" were employed.<sup>162, 163, 164</sup>

Adam *et al.*,<sup>166</sup> (CXLII) is regarded as the most probable configuration; <sup>167</sup> natural œstriol has been obtained from œstrone in 25% yield.<sup>165, 168</sup>



A *cis*-3 : 16 : 17-trihydroxyœstra-1 : 3 : 5-triene (CXLIII or CXLIV) has been synthesised <sup>167</sup> by hydroxylation of 3-benzoyloxyœstra-1 : 3 : 5 : 16-tetraene with osmium tetroxide and subsequent hydrolysis; since the rearward side of the relatively flat œstratetraene molecule is subject to less steric hindrance than the front face with the ( $\beta$ )-orientated C<sub>13</sub>-angular methyl group, the formula (CXLIII) is preferred. Huffmann and Lott <sup>165</sup> state that they have handled an œstriol (by implication different from *isocœstriol* A, *vide infra*), which very rapidly forms an acetonide; this may be identical with Ruzicka's epimeride (CXLIII) or may be (CXLIV). M. N. Huffmann and H. H. Darby <sup>169</sup> converted œstrone *via* the 16-*iso*-nitroso-compound,<sup>170</sup> reductive hydrolysis,<sup>171</sup> and hydrogenation of one of the resulting  $\alpha$ -ketols, into an epimeride (*isocœstriol* A), which is different from natural œstriol (CXLII) and from Ruzicka's epimeride (CXLIII); although no observations were made as to its capacity for acetonide formation, it may represent the fourth epimeride (CXLV).

3( $\beta$ )-Hydroxyandrost-16-ene by hydroxylation with osmium tetroxide <sup>172</sup> gives a *cis*-3( $\beta$ ) : 16 : 17-trihydroxyandrostane (as CXLIII); this is different from the epimeride obtained by hydrogenation of 3( $\beta$ ) : 16 : 17-trihydroxyandrost-5-ene isolated from urine,<sup>173, 174</sup> which has been synthesised from 3( $\beta$ )-hydroxyandrost-5-en-17-one *via* the 16-*isonitroso*-compound.<sup>165</sup> Application of optical superposition rules <sup>172</sup> shows that natural œstriol and the saturated triol from the natural androst-5-enetriol have the same configuration (CXLII) at C<sub>16</sub> and C<sub>17</sub>; this supports the view <sup>173, 175</sup> that *in vivo* œstriol is derived from œstrone and the urinary androst-5-enetriol from 3( $\beta$ )-hydroxyandrost-5-en-17-one (dehydro*iso*androsterone) by analogous processes.

C. W. S.

<sup>166</sup> N. K. Adam, J. F. Danielli, G. A. D. Haslewood, and G. F. Marrian, *Biochem. J.*, 1932, **26**, 1233.

<sup>167</sup> V. Prelog, L. Ruzicka, and P. Wieland, *Helv. Chim. Acta*, 1945, **28**, 250.

<sup>168</sup> M. N. Huffmann and W. R. Miller, *Science*, 1944, **100**, 312.

<sup>169</sup> *J. Amer. Chem. Soc.*, 1944, **66**, 150.

<sup>170</sup> F. Litvan and R. Robinson, *J.*, 1938, 1997.

<sup>171</sup> F. H. Stodola, E. C. Kendall, and B. F. McKenzie, *J. Org. Chem.*, 1941, **6**, 841.

<sup>172</sup> L. Ruzicka, V. Prelog, and P. Wieland, *Helv. Chim. Acta*, 1945, **28**, 1609.

<sup>173</sup> H. Hirschmann, *J. Biol. Chem.*, 1943, **150**, 363.

<sup>174</sup> G. F. Marrian and G. C. Butler, *Nature*, 1944, **154**, 19; *Biochem. J.*, 1944, **38**, 322.

<sup>175</sup> W. H. Pearlmann and G. Pincus, *J. Biol. Chem.*, 1943, **147**, 379.

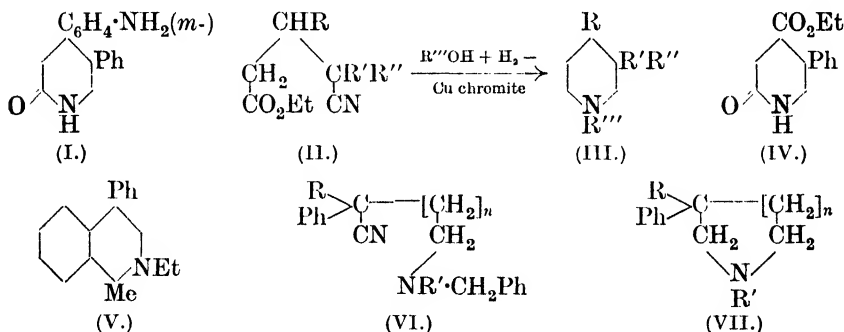


## 6. HETEROCYCLIC COMPOUNDS.

*Reduced Heterocyclic Rings.*

A. *Piperidines and Piperidones*.—Much of the recent work in this field has been inspired by the quest for analgesics modelled on morphine and pethidine (dolantin).

C. F. Koelsch's <sup>1</sup> synthesis of 3 : 4-disubstituted piperidines from  $\gamma$ -cyano-esters [*e.g.*, the reductive ring-closure of ethyl 3-cyano-3-phenyl-2-*m*-aminophenylbutyrate to the piperidone (I) <sup>2</sup>] has been modified by W. Barr and J. W. Cook,<sup>3</sup> who find that reduction of (II) by copper chromite in presence of an alcohol involves *N*-alkylation by the alcohol and leads to 1 : 3- or 1 : 3 : 4-substituted compounds (III). The following are examples of the conversion of (II) into (III) : (a)  $R = R' = \text{Ph}$ ,  $R'' = \text{H}$ ,  $R''' = \text{Me}$ ,  $\text{Et}$ ,  $\text{Bu}^a$ ; (b)  $R = \text{CO}_2\text{Et}$ ,  $R' = \text{Ph}$ ,  $R'' = \text{H}$ ,  $R''' = \text{Et}$  [this compound was accompanied by the piperidone (IV)]; and (c)  $R = \text{H}$ ;  $R' = [\text{CH}_2]_3 \cdot \text{OEt}$ ,  $R'' = \text{Ph}$ ,  $R''' = \text{Et}$  [from (II);  $R = \text{H}$ ,  $R' = \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CN}$ ,  $R'' = \text{Ph}$ ]]; the decahydroisoquinoline (V) was obtained from 2-acetylcyclohexylbenzyl cyanide. A formally similar ring-closure involving simultaneous debenzylation and elimination of ammonia was observed by F. Bergel and his co-workers.<sup>4</sup> Hydrogenolysis in presence of palladised charcoal of compounds of type (VI) gave rise to (VII), pyrrolidines ( $n = 1$ ) as well as piperidines



( $n = 2$ ) being obtainable by this reaction; examples of (VII) are  $n = 1$ ,  $R = \text{H}$ ,  $R' = \text{Me}$ ; and  $n = 1$  and  $2$ ,  $R = \text{CO}_2\text{Et}$ ,  $R' = \text{Me}$ . If  $R' = \text{CH}_2\text{Ph}$ , double debenzylation occurs, leading, for instance, to (VII,  $n = 2$ ,  $R = \text{CO}_2\text{Et}$ ,  $R' = \text{H}$ ).

The Eisleb <sup>5</sup> method of piperidine synthesis by double alkylation of a reactive methylene group with an alkylbis-2-chloroethylamine has been applied by several groups of workers. Thus aralkyl cyanides of type (VIII)

<sup>1</sup> *J. Amer. Chem. Soc.*, 1943, **65**, 2093; *Ann. Reports*, 1943, **40**, 163.

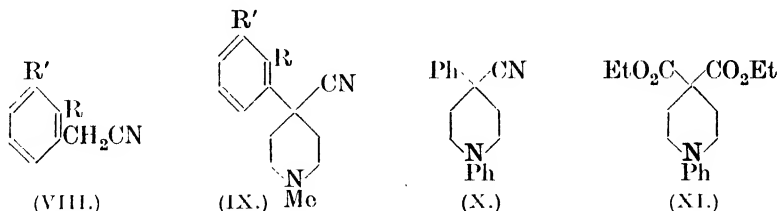
<sup>2</sup> C. F. Koelsch and R. F. Raffauf, *J. Amer. Chem. Soc.*, 1944, **66**, 1857.

<sup>3</sup> *J.*, 1945, 438.

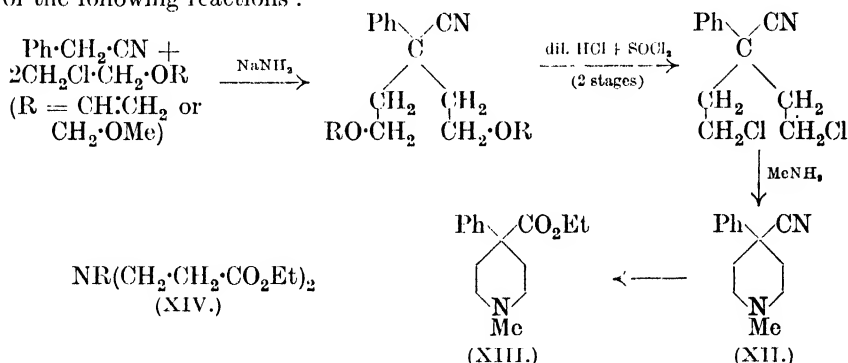
<sup>4</sup> F. Bergel, N. C. Hindley, A. L. Morrison, and H. Rinderknocht, *J.*, 1944, 269.

<sup>5</sup> O. Eisleb, *Ber.*, 1941, **74**, 1433; *Ann. Reports*, 1941, **38**, 199.

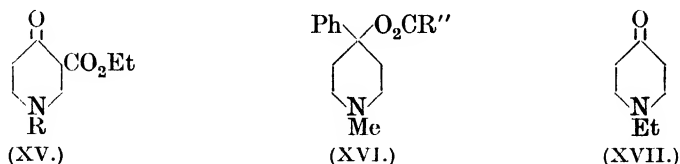
condense with methylbis-2-chloroethylamine to give the piperidines (IX; R = OMe; R' = H), (IX; R = O-CH<sub>2</sub>Ph; R' = H), and (IX; R = R' =



OMe),<sup>6</sup> the dolantin-type compounds (X) and (XI) have been prepared from phenylbis-2-chloroethylamine and benzyl cyanide and malonic ester respectively,<sup>7</sup> and a variation of the same alkylation method has been employed<sup>8</sup> in a new synthesis of dolantin (pethidine) itself (XIII) by means of the following reactions :



A well-established route which is still being further explored is the synthesis of *N*-substituted 4-piperidones from alkylbis-2-carbethoxyalkylamines (XIV). From (XIV; R = Me), K. A. Jensen and F. Lundquist<sup>9</sup> have prepared, *via* the intermediate piperidone (XV; R = Me), a series of compounds (XVI) [amongst them an isomer (XVI; R'' = Et) of dolantin]



by the obvious stages of hydrolysis and decarboxylation, Grignard reaction, and acylation. The method is an excellent preparative route for certain

<sup>6</sup> F. Bergel, J. W. Haworth, A. L. Morrison, and H. Rinderknecht, *J.*, 1944, 261; J. A. Barltrop, *J.*, 1946, 958.

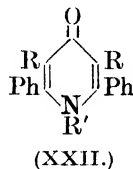
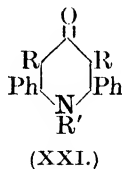
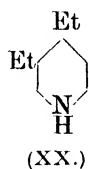
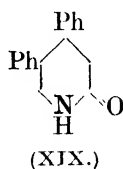
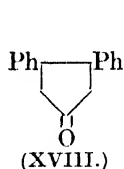
<sup>7</sup> R. M. Anker, A. H. Cook, and (Sir) I. M. Heilbron, *J.*, 1945, 917.

<sup>8</sup> F. Bergel, A. L. Morrison, and H. Rinderknecht, *J.*, 1944, 265.

<sup>9</sup> *Dansk Tidsskr. Farm.*, 1943, 17, 173.

compounds; thus 1-ethyl-4-piperidone (XVII) has been prepared in two stages (yields 94% and *ca.* 85%) from ethyl acrylate and ethylamine,<sup>10</sup> reduction of the ketone in presence of ammonia yielding 4-amino- and some 4-hydroxy-1-ethylpiperidine. The same route has also been used by D. R. Howton<sup>11</sup> to prepare 1:3-dimethyl-4-piperidone, and A. H. Cook and K. J. Reed<sup>12</sup> prepared 3-cyano-1-methyl-4-piperidone analogously (Thorpe reaction) from methylamine and vinyl cyanide. Further, S. M. McElvain and G. Stork<sup>13</sup> have shown that, in the reaction between ethyl acrylate and ammonia, an equilibrium is formed between the reactants and  $\text{NH}_2\text{R}$ ,  $\text{NHR}_2$ , and  $\text{NR}_3$  ( $\text{R} = \text{CH}_2\text{-CH}_2\text{-CO}_2\text{Et}$ ), but that, in presence of benzoyl chloride, (XIV;  $\text{R} = \text{Bz}$ ) is formed in high yield; application of the Dieckmann reaction then gives (XV;  $\text{R} = \text{Bz}$ ), which, through its facile debenzoylation, is a valuable intermediate for the synthesis of piperidones and piperidines unsubstituted on the nitrogen [ring-closure of (XIV;  $\text{R} = \text{H}$ ) is attended by deleterious side-reactions]. Examples of the usefulness of (XIV;  $\text{R} = \text{Bz}$ ) are given below.

C. F. Koelsch and his co-workers have studied the formation of stereoisomeric 4:5-substituted 2-piperidones by means of the Beckmann change applied to cyclopentanone oximes. Thus the *cis*- and *trans*-forms of (XVIII) yielded *cis*- and *trans*- (XIX), identical with samples prepared by deamination of the stereoisomers of (I).<sup>2</sup> The analogous diethyl derivatives were also synthesised, and from these piperidones *cis*- and *trans*-3:4-diethylpiperidine (XX) were prepared.<sup>14</sup> In this connexion it may be noted that the piperidones (XXI;  $\text{R} = \text{H}$ ;  $\text{R}' = \text{Me}$ ) and (XXI;  $\text{R} = \text{CO}_2\text{Et}$ ;  $\text{R}' = \text{Et}$ ) have each been isolated in stereoisomeric forms, each pair of



stereoisomers on oxidation yielding the same pyridone (XXII;  $\text{R} = \text{H}$ ;  $\text{R}' = \text{Me}$ ) and (XXII;  $\text{R} = \text{CO}_2\text{Et}$ ;  $\text{R}' = \text{Et}$ ).<sup>15</sup> 1-Alkyl-2:4- and -2:6-bis-2-arylethylpiperidines (as XXIII) have been prepared<sup>16</sup> by reduction of distyryl-1-alkylpyridinium *p*-toluenesulphonates.

The reactivity of *N*-alkenylpiperidines has been investigated by C. Manich and E. Kniss.<sup>17</sup> On distillation under nitrogen, (XXIV;  $\text{R} = \text{C}_5\text{H}_{11}$ )

<sup>10</sup> R. C. Fuson, W. E. Parham, and L. J. Reed, *J. Amer. Chem. Soc.*, 1946, **68**, 1239.

<sup>11</sup> *J. Org. Chem.*, 1945, **10**, 277.

<sup>12</sup> *J.*, 1945, 399.

<sup>13</sup> *J. Amer. Chem. Soc.*, 1946, **68**, 1049.

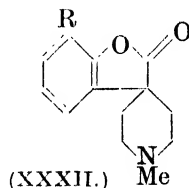
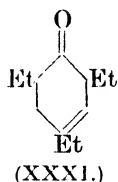
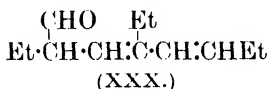
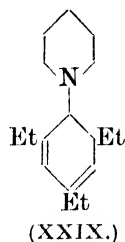
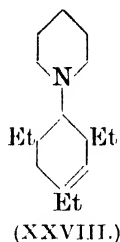
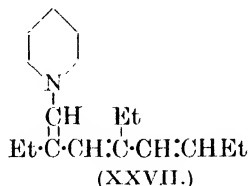
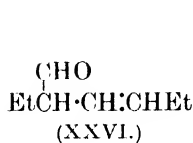
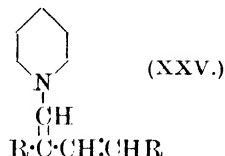
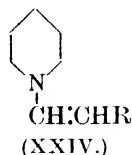
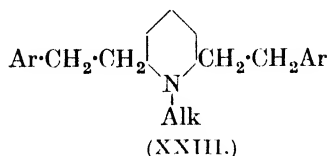
<sup>14</sup> C. F. Koelsch and C. H. Stratton, *ibid.*, 1944, **66**, 1881.

<sup>15</sup> P. I. Petrenko-Kritschenko and T. K. Tschumatschenko, *Compt. rend. Acad. Sci. U.R.S.S.*, 1940, **27**, 470.

<sup>16</sup> J. Lee and W. Freudenberg, *J. Org. Chem.*, 1944, **9**, 537.

<sup>17</sup> *Ber.*, 1941, **74**, 1629.

gives piperidine and (XXV;  $R = C_5H_{11}$ ). The simpler case (XXIV;  $R = Et$ ) was studied in greater detail. Thermal decomposition yielded



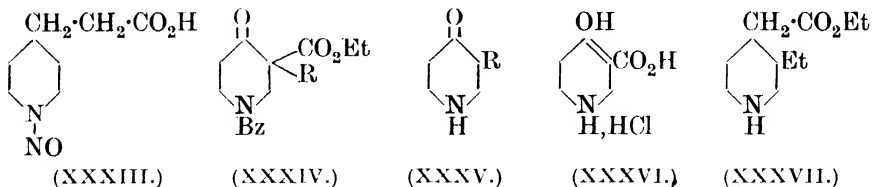
piperidine, (XXV;  $R = Et$ ) [hydrolysed to the aldehyde (XXVI)], and a mixture containing (XXVII), (XXVIII), and (XXIX). The structure of the last was proved by reduction to *N*-2 : 4 : 6-triethylcyclohexylpiperidine, and that of (XXVII) and of (XXVIII) by acid hydrolysis to (XXX) and (XXXI) respectively.

The reactivity of cyano-groups in *N*-substituted 4-cyano-4-arylpiperidines is frequently, but not always, normal. Thus (IX;  $R = OMe$ ;  $R' = H$ ) and (X) undergo normal Grignard reactions,<sup>6, 7</sup> and the expected hydrolyses occur with (IX;  $R = O \cdot CH_2Ph$ ;  $R' = H$ ), with (IX;  $R = R' = OMe$ ) [with production of the *isocoumaranones* (XXXII;  $R = H$  and  $OMe$ )],<sup>6</sup> with (X),<sup>7</sup> and with (XII).<sup>8</sup> On the other hand, attempts by F. Bergel *et al.* to convert such compounds into 4-aminomethyl derivatives by sodium and alcohol led invariably to elimination of the cyano-group and replacement by hydrogen,<sup>6</sup> a reaction which the authors consider may be characteristic of  $Ar \cdot CR_1R_2 \cdot CN$  compounds in general.

C. F. Koelsch<sup>18</sup> has drawn attention to the usefulness of *N*-nitrosation as a means of stabilising piperidines where this is desirable. For example, the great reactivity of 4-bromomethylpiperidine *per se* excludes its use in malonic ester condensations; the *N*-nitroso-derivative, however, yields 1-nitroso-4-piperidylmethylmalonic acid, and thence the  $\beta$ -propionic acid (XXXIII). *N*-Benzoylation has the added advantage of facilitating

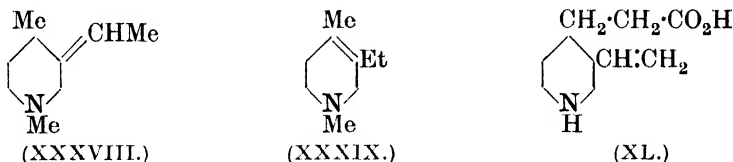
<sup>18</sup> *J. Amer. Chem. Soc.*, 1944, **66**, 1611.

removal at a later stage of the protecting group, yielding derivatives not otherwise accessible. Thus 3-alkylation of (XV;  $R = \text{Me}$ ) is accompanied



by quaternisation, but with (XV;  $R = \text{Bz}$ ) the reaction proceeds smoothly yielding the 3-alkyl derivatives (XXXIV;  $R = \text{Et}$  and  $\text{CH}_2\text{Ph}$ ), whence the 3-alkyl-4-piperidones (XXXV) are readily obtained.<sup>19</sup> Reduction, hydrolysis, and dehydration of (XV;  $R = \text{Bz}$ ) gives guvacine hydrochloride (XXXVI).<sup>13</sup> 4-Piperidones undergo the Knoevenagel reaction in presence of ammonium acetate;<sup>19</sup> in this way the ethyl ester of *dl*-cinchoiloipon (XXXVII) (the groups are assumed to be *cis*-) was synthesised from 1-benzoyl-3-ethyl-4-piperidone and ethyl cyanoacetate.

V. Prelog and E. Moor<sup>20</sup> were unsuccessful in attempts to make 3-vinylpiperidines from 1:4-dimethyl-3-1'-hydroxyethylpiperidine; the only products obtained by various methods of dehydration were the ethylidene derivative (XXXVIII) and the tetrahydropyridine (XXXIX), and the authors conclude that earlier compounds of this type<sup>21</sup> are not in fact vinyl



derivatives. It appears that the only case of a genuine synthesis of such a compound is R. B. Woodward and W. E. Doering's<sup>22</sup> synthesis of 2-(3-vinyl-4-piperidyl)propionic acid (homomeroquinene, XL).

**B. Bicyclic Compounds.**—(i) *cycloAlkano-pyrrolidines, -piperidines, and -thiazoles*. V. Prelog and his co-workers<sup>23, 24</sup> have applied the following reaction series to the synthesis of *cyclopentano*- and *cycloheptano*-2:3-pyrrolidine (XLIII;  $n = 3$  and 5). The compound (XLIII;  $n = 3$ ) was obtained in only one stereoisomeric form, but reduction of the oxime of (XLII;  $n = 5$ ) gave two stereoisomeric amines, from which *cis*- and *trans*-*cycloheptano*-2:3-pyrrolidine were prepared.

The synthesis of the piperidines (XLV;  $n = 3, 4$ , and 5) by the following

<sup>19</sup> G. Stork and S. M. McElvain, *J. Amer. Chem. Soc.*, 1946, **68**, 1053.

<sup>20</sup> *Helv. Chim. Acta*, 1945, **28**, 182.

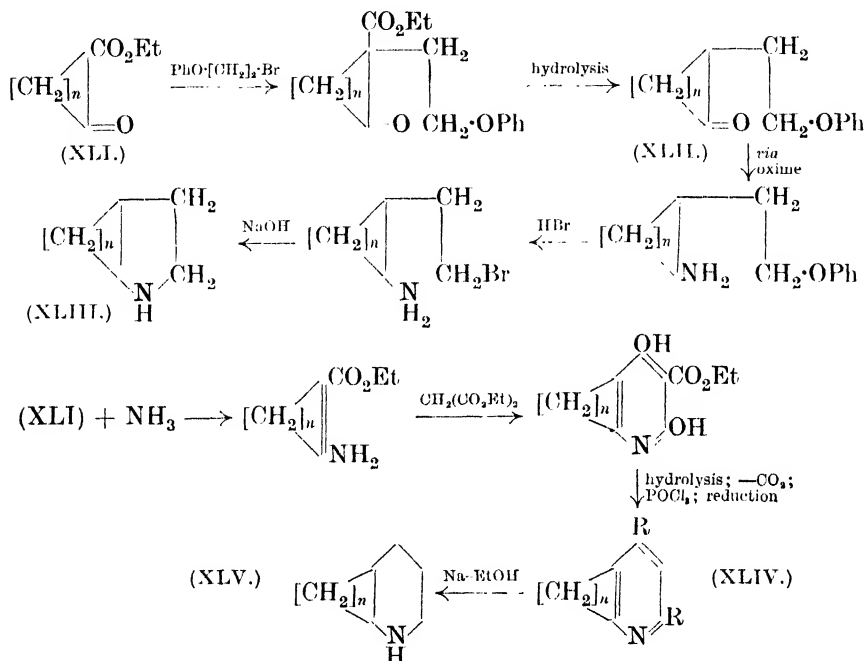
<sup>21</sup> H. A. Iddles, E. H. Lang, and D. C. Gregg, *J. Amer. Chem. Soc.*, 1937, **59**, 1945.

<sup>22</sup> *Ibid.*, 1945, **67**, 860.

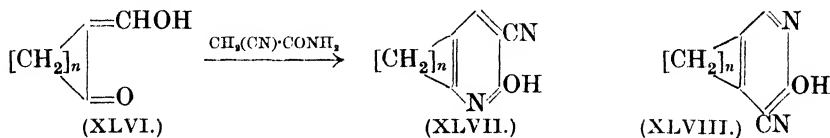
<sup>23</sup> V. Prelog and S. Szpilfogel, *Helv. Chim. Acta*, 1945, **28**, 178.

<sup>24</sup> V. Prelog and U. Geyer, *ibid.*, p. 576.

route has also been reported from the same laboratory.<sup>25, 26</sup> Pyridan (XLIV; R = H;  $n = 3$ ) and *Bz*-tetrahydroquinoline (XLIV; R = H;



$n = 4$ ) had previously been synthesised,<sup>27, 28</sup> but the method employed, *viz.*, condensation of (XLVI) with cyanoacetamide to give (XLVII), followed by the obvious transformations, did not exclude the possibility that the primary product might have been (XLVIII); the malonic ester synthesis, however, is unambiguous. The latter method was unsatisfactory for the synthesis of (XLV;  $n = 13$ ); this substance was, however, obtained *via* the cyanoacetamide method.<sup>29</sup> Both *cis*- and *trans*-forms of (XLV;  $n = 13$ ) were obtained according to the reduction conditions used; using the reagents



shown above, (XLV;  $n = 3, 4$ , and  $5$ ) each gave only a single stereoisomer. The conventional configurations (Auwers-Skita rule) are assigned to these

<sup>25</sup> V. Prelog and W. Hinden, *Helv. Chim. Acta*, 1944, **27**, 1854.

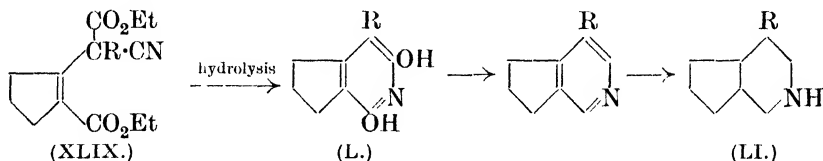
<sup>26</sup> V. Prelog and S. Szpilfogel, *ibid.*, 1945, **28**, 1684.

<sup>27</sup> W. C. Thompson, *J. Amer. Chem. Soc.*, 1931, **53**, 3160.

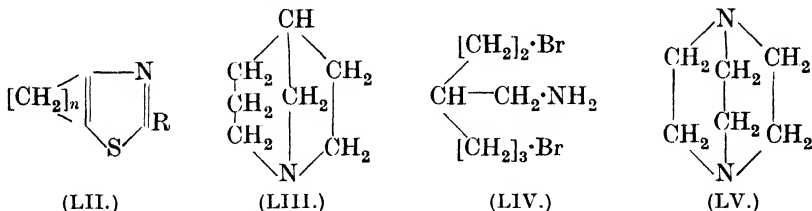
<sup>28</sup> J. von Braun and G. Lemke, *Annalen*, 1930, **478**, 182.

<sup>29</sup> V. Prelog and U. Geyer, *Helv. Chim. Acta*, 1945, **28**, 1677.

products.<sup>26,29</sup> It may be noted that the authors<sup>26</sup> dispute the claim of G. R. Clemo, J. G. Cook, and R. Raper<sup>30</sup> that *cis*- is converted into *trans*-decahydroquinoline by treatment with acid. The 3:4-piperidine derivatives (LI; R = H and Et) have also been synthesised<sup>31,32</sup> by the same procedure from the intermediates (XLIX; R = H and Et) [from (XLI;  $n = 3$ ) and ethyl cyanoacetate];<sup>33</sup> (L; R = H) was also obtained by the alternative route from (XLI;  $n = 3$ ) and cyanoacetamide.<sup>34</sup>



In this connexion it may be noted that thiazoles of type (LII; R = NH<sub>2</sub>;  $n = 3, 4, 5$ , and 6) have been prepared (from cyclic  $\alpha$ -chloro-ketones and thioamides) by H. Erlenmeyer and W. Schoenhauer<sup>35</sup> in order to study the Mills-Nixon effect in heterocyclic compounds. The cyclopentane derivative (LII; R = NH<sub>2</sub>;  $n = 3$ ) showed marked chemical, optical (absorption spectra), and biological differences from the homologue ( $n = 4$ ). Similar optical differences, but less pronounced, were also observed between the thiazoles (LII; R = H;  $n = 3$ ) and (LII; R = H;  $n = 4$ ).<sup>36</sup> It is of interest that (XLIV; R = OH;  $n = 3$ ) and (XLIV; R = OH;  $n = 4$ ) also show some difference in intensity of absorption, but in the opposite sense from the thiazole derivatives, *i.e.*, the intensity is higher for  $n = 3$  than for  $n = 4$  in the pyridine compounds.<sup>26</sup>



(ii) bicycloAza-alkanes. Using a method previously developed,<sup>37</sup> V. Prelog and K. Balenovic<sup>38</sup> have synthesised the unsymmetrical bicyclo-[2:3:1]-1-azaoctane (LIII) *via* the dibromoamine (LIV). bicyclo-[2:2:2]-1:4-Diazoctane (LV) is produced by heating the hydrochlorides of diethanolamine, a mixture of mono- and di-ethanolamine, or triethanolamine and ammonium chloride;<sup>39</sup> the best preparative method is pyrolysis of the

<sup>29</sup> *J.*, 1938, 1183.

<sup>31</sup> V. Prelog and O. Mitzler, *Helv. Chim. Acta*, 1946, **29**, 1163.

<sup>32</sup> *Idem*, *ibid.*, p. 1170.

<sup>33</sup> G. A. R. Kon and H. R. Nanji, *J.*, 1932, 2426.

<sup>34</sup> Cf. H. K. Sen and U. Bose, *J. Indian Chem. Soc.*, 1927, **4**, 51.

<sup>35</sup> *Helv. Chim. Acta*, 1941, **24**, 172E.

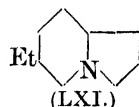
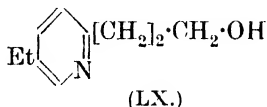
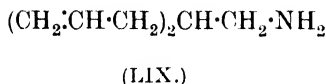
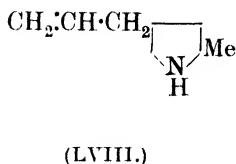
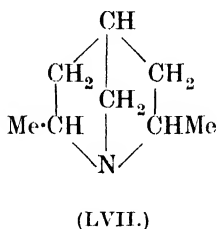
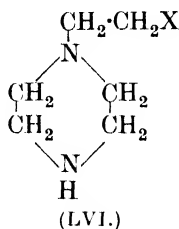
<sup>36</sup> H. Erlenmeyer and G. Bischoff, *ibid.*, 1946, **29**, 280.

<sup>37</sup> *Ann. Reports*, 1942, **39**, 200.

<sup>38</sup> *Ber.*, 1941, **74**, 1510.

<sup>39</sup> O. Hromatka, *ibid.*, 1942, **75**, 1302.

hydrohalide salts of 1-2'-chloro- or -bromo-ethylpiperazine (LVI; X = Cl or Br).<sup>40</sup> The dimethylbicycloazaheptane (LVII) is produced, together with 2-methyl-4-allylpyrrolidine (LVIII), by the action of hydrobromic acid and alkali on 2 : 2-diallylethylamine (LIX).<sup>41</sup> The synthesis of 6-ethylindolizidine (LXI) is reported by V. Prelog and O. Mitzler<sup>31</sup> from (LX) [from 2-lithium-methyl-5-ethylpyridine and ethylene oxide] by reduction, replacement of hydroxyl by bromine, and alkaline cyclisation.



C. *The β-Biotin Problem.*—Work which has appeared since the last review in these reports<sup>42</sup> has been mainly directed towards the elucidation of the steric configuration of β-biotin (LXII). Of the four racemates which can theoretically arise from the presence of three asymmetric centres (●) in (LXII), the three previously isolated by Harris and his co-workers<sup>43</sup>—*dl*-biotin, *dl*-allobiotin, and *dl*-epiallobiotin—have been studied from this aspect in some detail.<sup>44</sup> It was shown that each of the alternative routes (LXIII) → (LXIV) → (LXII) → (LXV) and (LXIII) → (LXVI) → (LXVII) → (LXV) gave rise to the same dethiobiotin (LXV) when applied to a given racemic bisacylamidothiophan derivative (LXIII); in this way *dl*-allo-(LXIII) and *dl*-epiallo-(LXIII) both gave *dl*-dethioallo-biotin (biologically inactive), and *dl*-(LXIII) [the precursor of *dl*-biotin, of which natural (*d*-)β-biotin is one component] gave *dl*-dethiobiotin (biologically active), thus confirming that, in the three racemates (LXII), two have a *trans*-linkage at C<sub>3</sub>–C<sub>4</sub> and one has a *cis*-, or *vice versa*. Now in the reaction (LXIV) → (LXII), the yield is almost theoretical in the case of *dl*-biotin, but this does not apply to the *dl*-allo- and *dl*-epiallo-compounds. This suggests that *dl*-biotin has the *cis*-configuration at C<sub>3</sub>–C<sub>4</sub> (LXVIII), and that the *dl*-allo- and *dl*-epiallo-racemates are *trans*-compounds (LXIX). This is strikingly confirmed by the behaviour of the three racemates towards boiling

<sup>40</sup> O. Hromatka and E. Engel, *Ber.*, 1943, **76**, 712.

<sup>41</sup> R. Paul and H. Cottin, *Bull. Soc. chim.*, 1940, **7**, 626.

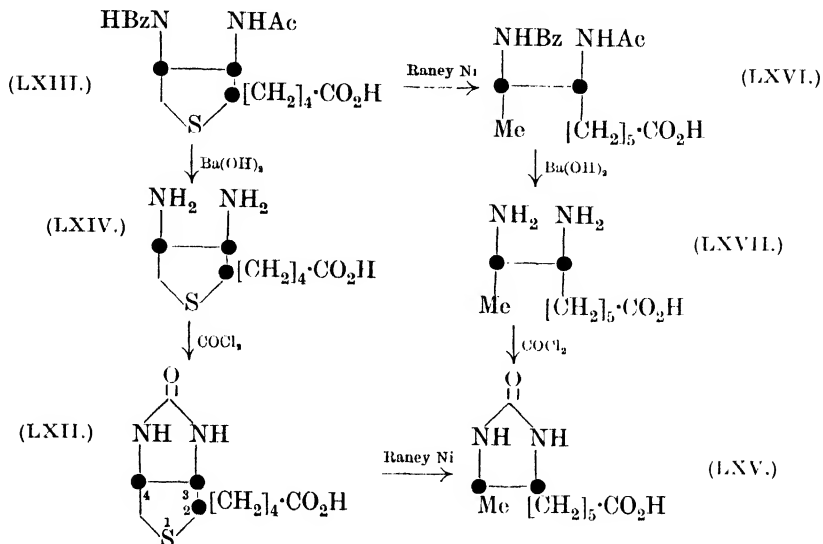
<sup>42</sup> *Ann. Reports*, 1944, **41**, 217.

<sup>43</sup> S. A. Harris, D. E. Wolf, R. Mazingo, G. E. Arth, R. C. Anderson, N. R. Easton, and K. Folkers, *J. Amer. Chem. Soc.*, 1945, **67**, 2090.

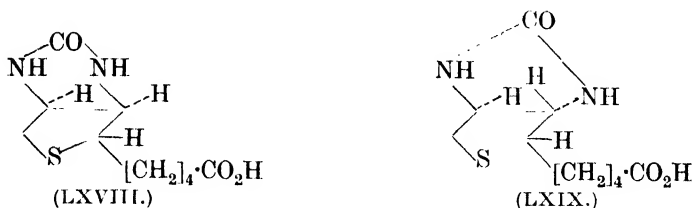
<sup>44</sup> S. A. Harris, R. Mazingo, D. E. Wolf, A. N. Wilson, and K. Folkers, *ibid.*, p. 2102.



dilute sulphuric acid; *dl*-biotin is unaffected, whereas the *dl*-allo- and *dl*-epiallo-racemates are hydrolysed to carbon dioxide and the corresponding



diamino-acid (LXIV). The absolute configurations about  $C_2$  for the racemates have not yet been determined. Of the three reagents (*d*-mandelic



acid, quinidine methohydroxide, and *L*-arginine) which have been used<sup>45</sup> for the resolution of *dl*-biotin, the last is most suitable for isolation of the natural enantiomorph.

A. Grüssner, J.-P. Bourquin, and O. Schnider<sup>46</sup> have also synthesised *dl*-biotin by the following route; the esters (LXX;  $R = \text{Me}$  or  $\text{Et}$ ;  $R' = [\text{CH}_2]_4\cdot\text{OMe}$ ) were prepared by the method of H. Schmid<sup>47</sup> (see following page). In this synthesis, the separation of individual racemates commenced at (LXXI); *dl*- $\beta$ -biotin having properties in excellent agreement with those described by the American workers<sup>43,44</sup> was thus obtained.<sup>46,48</sup> Two other substances were also isolated<sup>46</sup> in this work; however, these products were

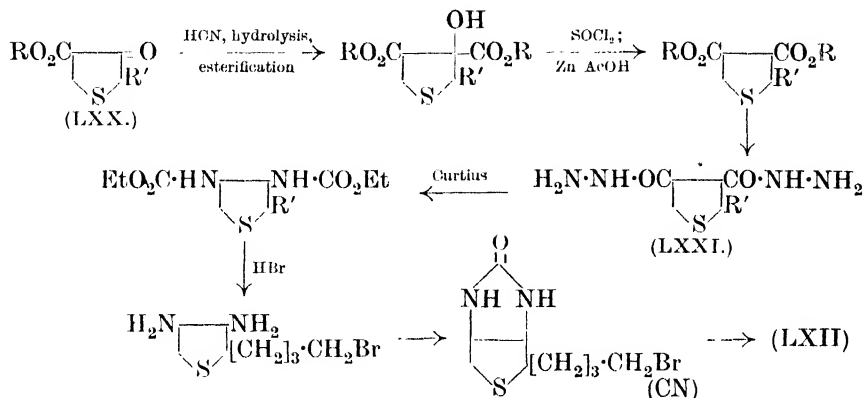
<sup>45</sup> D. E. Wolf, R. Mazingo, S. A. Harris, R. C. Anderson, and K. Folkers, *J. Amer. Chem. Soc.*, 1945, **67**, 2100.

<sup>46</sup> *Helv. Chim. Acta*, 1945, **28**, 517.

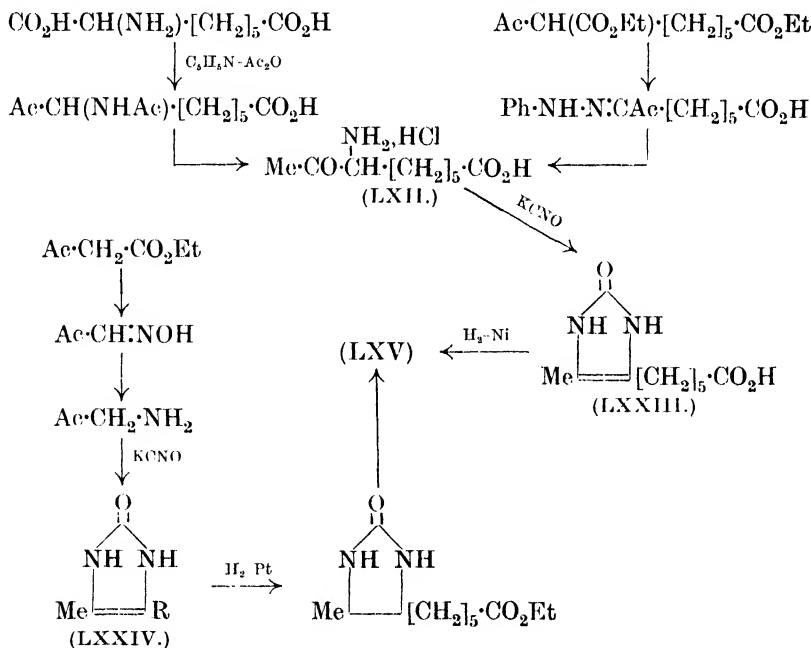
<sup>47</sup> H. Schmid, *ibid.*, 1944, **27**, 127.

<sup>48</sup> A. Grüssner, J.-P. Bourquin, and O. Schnider, *ibid.*, 1946, **29**, 770.

not identical with *dl-allo-* or *dl-epiallo-*biotin, and the claim <sup>46</sup> that they were pure racemates has lately been withdrawn.<sup>48</sup>



Several papers have appeared dealing with syntheses of dethiobiotin (LXV). J. L. Wood and V. du Vigneaud,<sup>49</sup> utilising the Dakin-West reaction,<sup>50</sup> prepare  $\omega$ -amino- $\omega$ -acetylheptoic acid (LXXII) from 1-amino-



suberic acid, whence the synthesis proceeds on conventional lines. The final stage gave a mixture of stereoisomers which was not resolved. The

<sup>49</sup> J. Amer. Chem. Soc., 1945, **67**, 210.

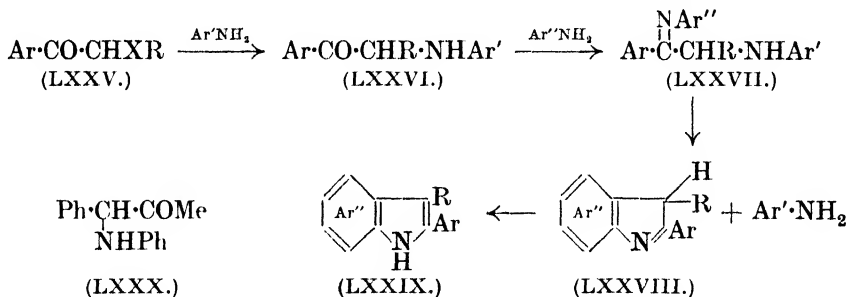
<sup>50</sup> H. D. Dakin and R. West, J. Biol. Chem., 1928, **78**, 91.

synthesis evolved by J.-P. Bourquin, O. Schnider, and A. Grüssner<sup>51</sup> is similar to that of Wood and du Vigneaud,<sup>49</sup> except that the  $\omega$ -amino- $\omega$ -acetylheptioic acid was prepared from 1-acetylsuberic ester by the Japp-Klingemann reaction; the dethiobiotin of these workers was later<sup>48</sup> recognised to be a mixture of *dl*-dethiobiotin and *dl*-dethioallobiotin, both components having been isolated. The synthesis by R. Duschinsky and L. A. Dolan<sup>52</sup> uses a different approach. These workers observed that 2-keto-4-methyl-2:3-dihydroglyoxaline (LXXIV; R = H), obtainable by the route \* shown or from the ester (LXXIV; R = CO<sub>2</sub>Et) by hydrolysis, can be acylated at C<sub>5</sub> under Friedel-Crafts conditions. Catalytic reduction of [LXXIV; R = CO·[CH<sub>2</sub>]<sub>4</sub>·CO<sub>2</sub>Et], followed by hydrolysis, led to *dl*-dethiobiotin, the physical constants and biological activity of which are in excellent agreement with those of material obtained<sup>44</sup> from (LXII) or (LXIII).

These results are an interesting illustration of the Auwers-Skita rule—reduction of (LXXIII) or its methyl ester in neutral or alkaline solution<sup>49, 51</sup> (with a nickel catalyst) failed to give a sterically homogeneous product, whereas reduction of the esters (LXXIV; R = CO·[CH<sub>2</sub>]<sub>4</sub>·CO<sub>2</sub>Et or [CH<sub>2</sub>]<sub>5</sub>·CO<sub>2</sub>Et) in acid solution with Adams's catalyst led to complete *cis*-addition.<sup>52</sup>

#### Indoles.

The synthesis of indoles from arylamines and  $\alpha$ -halogeno- or  $\alpha$ -hydroxyketones, of which the reaction using phenacylarylamines is a special case, has been comprehensively studied by Julian and his co-workers.<sup>53</sup> These authors disagree with recent criticisms<sup>54, 55</sup> which reject the assumption



of the "amine-anil" stage (LXXXVII) inherent in the Bischler mechanism [(LXXXV; X = OH or halogen)  $\rightarrow$  (LXXXVI)  $\rightarrow$  (LXXXVII)  $\rightarrow$  (LXXXVIII)  $\rightarrow$  (LXXIX)]. The American workers have shown that the reaction of (LXXXV;

<sup>51</sup> *Helv. Chim. Acta*, 1945, **28**, 528.

<sup>52</sup> *J. Amer. Chem. Soc.*, 1945, **67**, 2079.

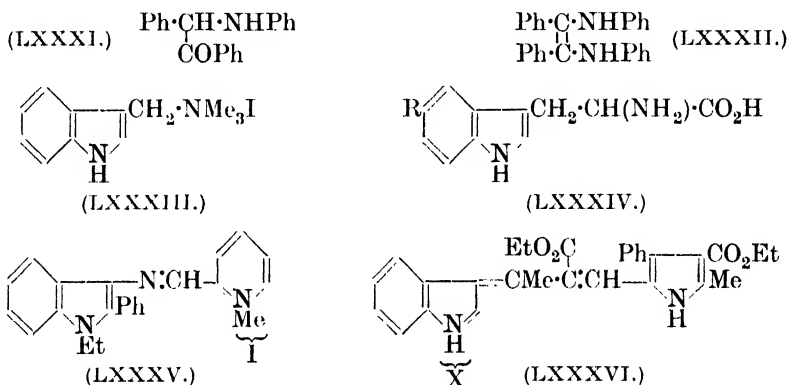
<sup>53</sup> P. L. Julian, E. W. Meyer, A. Magnani, and W. Cole, *ibid.*, p. 1203.

<sup>54</sup> A. F. Crowther, F. G. Mann, and D. Purdie, *J.*, 1943, 58; *Ann. Reports*, 1943, **40**, 160.

<sup>55</sup> P. E. Verkade and E. F. J. Janetsky, *Rec. Trav. chim.*, 1943, **62**, 763, 775.

\* This route has been utilised by H. McKennis and V. du Vigneaud (*J. Amer. Chem. Soc.*, 1946, **68**, 832) in the production of a series of esters, CO<sub>2</sub>Et·[CH<sub>2</sub>]<sub>n</sub>·CHAc·NH<sub>2</sub>, from which analogues of dethiobiotin were prepared.

X = Br) with aniline is not unidirectional; for example,  $\alpha$ -bromopropiophenone gives not only (LXXVI; Ar = Ar' = Ph; R = Me), but also  $\alpha$ -anilinobenzyl methyl ketone (LXXX), and  $\alpha$ -bromo- $\beta$ -phenylpropiophenone (LXXV; Ar = Ph; X = Br; R = CH<sub>2</sub>Ph) behaves similarly.<sup>56</sup> Further, an intimate study of the conversion of desylaniline (LXXXI) into 2:3-diphenylindole in presence of aniline and hydrochloric acid led to the isolation of the anil (LXXXII or its tautomer—"amine-anil"), which was separately converted into 2:3-diphenylindole; in agreement with these results, no reaction occurred when dimethylaniline was used instead of aniline. The authors contend that the assumptions of dissociation of (LXXVI), followed by rearrangement or recombination with a second amine residue,<sup>54, 55</sup> are thus invalidated; and they conclude that the Bischler mechanism is substantially correct with the qualifications (a) that isomers are formed at the stage (LXXV)  $\rightarrow$  (LXXVI), and (b) that the aryl group



eliminated as arylamine from (LXXVII) depends on the nature of (LXXVII) and not on the order of insertion into its molecule of the two arylamine residues.

The recent syntheses of tryptophan\* from gramine methiodide (LXXXIII)<sup>57</sup> (based on the *C*-alkylating properties of quaternary salts containing a benzyl group)<sup>58</sup> have been still further improved; the reaction is conveniently effected by quaternising the gramine *in situ*,<sup>59, 60</sup> and the use of ethyl acetamidocyanoacetate instead of ethyl acylamidomalonates gives pure (LXXXIV; R = H) in 71% yield<sup>60</sup> from crude indole; 5-methyl-

<sup>56</sup> S. N. McGeoch and T. S. Stevens, *J.*, 1935, 1032.

<sup>57</sup> H. R. Snyder and C. W. Smith, *J. Amer. Chem. Soc.*, 1944, **66**, 350; N. F. Albertson, S. Archer, and C. M. Suter, *ibid.*, p. 500; *Ann. Reports*, 1944, **41**, 123.

<sup>58</sup> H. R. Snyder, C. W. Smith, and J. M. Stewart, *J. Amer. Chem. Soc.*, 1944, **66**, 200.

<sup>59</sup> N. F. Albertson, S. Archer, and C. M. Suter, *ibid.*, 1945, **67**, 36.

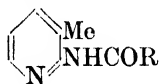
<sup>60</sup> N. F. Albertson and B. F. Tullar, *ibid.*, p. 502.

\* For improvements of earlier methods, see J. Elks, D. F. Elliott, and B. A. Hems, *J.*, 1944, 629. B. Hegedüs (*Helv. Chim. Acta*, 1946, **29**, 1499) describes a synthesis of tryptophan which involves the Fischer reaction and avoids the direct use of indole.

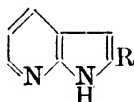
tryptophan (LXXXIV;  $R = \text{Me}$ ) is similarly prepared.<sup>61</sup> Owing to the great reactivity of the dimethylaminomethyl group in gramine it is even possible to achieve the condensation with the unquaternised base.<sup>62</sup>

The preparation of indole-3-aldehyde has been much simplified and improved by direct reaction between indole and *N*-methylformanilide,<sup>63</sup> and its condensation reactions have been studied.<sup>64</sup> Various condensations have been effected with 1:2-disubstituted 3-nitroso-indoles, from which, *inter alia*, a new class of cyanine dye (as LXXXV) can be prepared;<sup>65</sup> indole trimethincyanines, *e.g.*, (LXXXVI), have also been obtained from indoles capable of tautomerism to indolenines.<sup>66</sup>

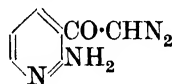
G. R. Clemo and G. A. Swan<sup>67</sup> have prepared 7-azaindole and its 2-methyl and 2-ethyl derivatives (LXXXVIII;  $R = \text{H}$ ,  $\text{Me}$ , and  $\text{Et}$ ) from the appropriate 2-acylamido-3-picolines (LXXXVII;  $R = \text{H}$ ,  $\text{Me}$ , and  $\text{Et}$ ), using sodium ethoxide as the condensing agent; the yields were low, and it is to be noted that potassium *tert.*-butoxide appears to be a superior reagent in the analogous preparation of 3-methyl- and 2:3-dimethyl-indole.<sup>68</sup> The oxindole, isatin, and indigo related to (LXXXVIII;  $R = \text{H}$ )



(LXXXVII.)



(LXXXVIII.)



(LXXXIX.)

have been prepared from 2-amino-3-diazoacetylpyridine (LXXXIX).<sup>69</sup> B. Witkop and G. Graser<sup>70</sup> find that ozonisation of indoles occurs best in formamide and involves fission at  $\text{C}_2\text{--C}_3$  with formation of the expected products. E. F. J. Janetsky and M. C. Lebreton<sup>71</sup> discuss the relationship between dipole moment and structure of substituted indoles.

*Gliotoxin*.—This crystalline antibiotic, which possesses powerful bacteriostatic action against various (especially Gram-positive) micro-organisms,<sup>72, 73</sup> is elaborated by *Gliocladium fimbricatum*,<sup>73</sup> an unidentified species of *Penicillium*,<sup>74</sup> and *Aspergillus fumigatus*.<sup>75</sup> Its structure has been largely elucidated by J. R. Johnson and his collaborators. The substance, of

<sup>61</sup> M. E. Jackman and S. Archer, *J. Amer. Chem. Soc.*, 1946, **68**, 2105.

<sup>62</sup> E. E. Howe, A. J. Zambito, H. R. Snyder, and M. Tishler, *ibid.*, 1945, **67**, 38.

<sup>63</sup> A. C. Shabica, E. E. Howe, J. B. Ziegler, and M. Tishler, *ibid.*, 1946, **68**, 1156.

<sup>64</sup> R. B. van Order and H. G. Lindwall, *J. Org. Chem.*, 1945, **10**, 128.

<sup>65</sup> F. G. Mann and R. C. Haworth, *J.*, 1944, 670.

<sup>66</sup> A. H. Cook and J. R. Majer, *J.*, 1944, 486.

<sup>67</sup> *J.*, 1945, 603.

<sup>68</sup> L. Marion and W. R. Ashford, *Canad. J. Res.*, 1945, **23**, B, 26.

<sup>69</sup> H. Kägi, *Helv. Chim. Acta*, 1941, **24**, 141E.

<sup>70</sup> *Annalen*, 1944, **556**, 103.

<sup>71</sup> *Rec. Trav. chim.*, 1944, **63**, 123.

<sup>72</sup> S. A. Waksman and H. B. Woodruff, *J. Bact.*, 1942, **44**, 373.

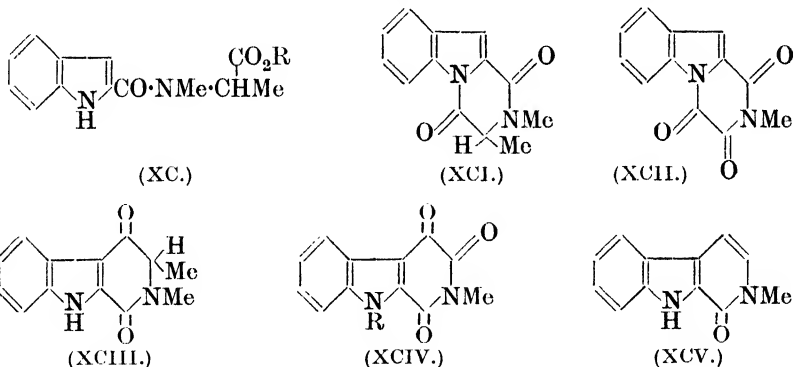
<sup>73</sup> J. R. Johnson, W. F. Bruce, and J. D. Dutcher, *J. Amer. Chem. Soc.*, 1943, **65**, 2005.

<sup>74</sup> J. R. Johnson, W. C. McCrone, jun. and W. F. Bruce, *ibid.*, 1944, **66**, 501.

<sup>75</sup> A. E. O. Menzel, O. Wintersteiner, and J. C. Hoogerheide, *J. Biol. Chem.*, 1944, **152**, 419; G. A. Glistler and T. I. Williams, *Nature*, 1944, **153**, 651.

formula  $C_{13}H_{14}O_4N_2S_2$ ,<sup>73</sup> is laevorotatory, neutral, and inert towards carbonyl reagents;<sup>76</sup> it yields diacyl derivatives, and is readily attacked by permanganate and hypochlorite with oxidation of the sulphur to sulphate.<sup>76</sup> Treatment with hot alkali yields methylamine, hydrogen sulphide, and indole-2-carboxylic acid.<sup>76</sup> Reduction with hydriodic acid yields a compound,  $C_{13}H_{12}O_2N_2$  (A), which is converted into (XC; R = H) by controlled hydrolysis. The corresponding ester (XC; R = Et), synthesised from indole-2-carboxyl chloride and the ethyl ester of *dl*-*N*-methylalanine, readily yields the precursor (A) with loss of alcohol.<sup>77</sup> A closely-related compound (B),  $C_{12}H_8O_3N_2$ , is formed when gliotoxin is heated with selenium to about 250°; hydrolysis of (B) gives the *N*-methylamide of indole-2-carboxylic acid, reconverted into (B) by condensation with ethoxalyl chloride.<sup>78</sup>

It seemed likely that, in the syntheses of (A) and (B), ring-closure had occurred on the NH group of the indole nucleus, leading to structures (XCI) and (XCII) respectively; cyclisation involving the 3-position [leading to (XCIII) and (XCIV; R = H) for (A) and (B)] could not, however, be excluded. An unequivocal decision as to the structure of (B) was reached in the following way.<sup>79</sup> Condensation of indole-2-carboxyl chloride with methylaminoacetaldehyde dimethylacetal is known to involve cyclisation



on  $C_3$ ,<sup>80</sup> giving (XCV); this on oxidation with chromic anhydride yielded (XCIV; R = H), which was not identical with compound (B) from gliotoxin; on the other hand (XCIV; R = Me), prepared by the same method, was identical with material obtained by the alternative synthesis from (XCVI) and ethoxalyl chloride. It is therefore certain that (B) has the structure (XCII) and not (XCIV; R = H). The structure (XCI) for (A),

<sup>76</sup> W. F. Bruce, J. D. Dutcher, J. R. Johnson, and L. L. Miller, *J. Amer. Chem. Soc.*, 1944, **66**, 614.

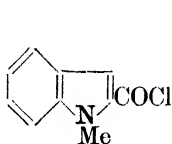
<sup>77</sup> J. D. Dutcher, J. R. Johnson, and W. F. Bruce, *ibid.*, p. 617.

<sup>78</sup> *Idem*, *ibid.*, p. 619.

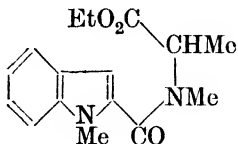
<sup>79</sup> J. R. Johnson, R. B. Hasbrouck, J. D. Dutcher, and W. F. Bruce, *ibid.*, 1945, **67**, 423.

<sup>80</sup> W. O. Kermack, W. H. Perkin, and R. Robinson, *J.*, 1922, **121**, 1872.

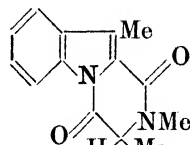
now virtually certain, was further supported by the failure of (XCVII), with a blocked 1-position, to undergo cyclisation [in contrast to the ease



(XCVI.)



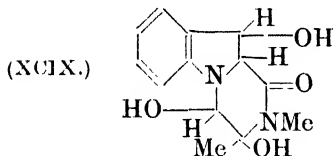
(XCVII.)



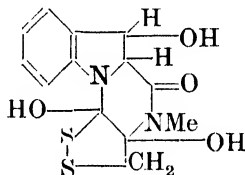
(XCVIII.)

with which (A) is formed from (XC; R = Et)], and by the close similarity between the ultra-violet absorption spectra of (A) and (XCVIII).

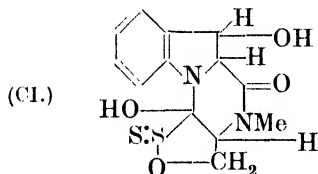
Considerable evidence in support of the presence of a disulphide linkage in gliotoxin is forthcoming from its instability to alkali, and the formation of lead sulphide on treatment with alkaline sodium plumbite, of an unstable adduct with potassium sulphide, and of an unstable dihydro-derivative (thiol compound) on treatment with potassium thioacetate.<sup>81</sup> That this linkage involves a potential methyl group ( $-C-CH_2-S-S-$ ) is indicated by the behaviour of dethiogliotoxin,  $C_{13}H_{16}O_4N_2$ , which is formed by mild reduction of gliotoxin; the dethio-compound gives approximately 1 mol. of acetic acid (Kuhn-Roth oxidation), whereas gliotoxin contains no C-methyl



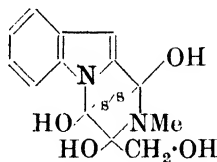
(XCIX.)



(C.)



(CI.)



(CII.)

group.<sup>81</sup> Dethiogliotoxin is formulated as (XCIX) on the basis of its hydrolysis to (XC; R = H), and the antibiotic itself is regarded as, most probably, (C); less likely alternatives are the indole hydrate corresponding to the hydroxyindoline (C), (CI), and (CII).<sup>81</sup>

### Quinolines.

The chief advance in this field since the last Report<sup>82</sup> has been the consolidation of preparative chemistry; for many classes of quinoline derivatives existing methods of preparation have been improved, and new

<sup>81</sup> J. D. Dutcher, J. R. Johnson, and W. F. Bruce, *J. Amer. Chem. Soc.*, 1945, **67**, 1736.

<sup>82</sup> *Ann. Reports*, 1943, **40**, 164.

methods developed. Much of this has emerged from the programme of antimalarial research carried out in the United States; in this work special attention has been paid to the synthesis of 4-hydroxy- and 4-aminoalkyl-amino-quinolines (the latter from the former by standard methods). In nearly all the methods (outlined below) for the preparation of 4-hydroxy-quinolines, high-temperature cyclisation is employed, and an outstanding feature of this work is the marked improvement resulting from the use of boiling diphenyl, or a mixture of this and diphenyl ether ("Dowtherm A"), as cyclisation medium. In the following summary of methods, reference is made only to the latest papers, and not to the earlier literature on which the new modifications (usually of the Conrad-Limpach synthesis) are largely based.

(1) Cyclisation of  $\text{Ar}\cdot\text{N}\cdot\text{CR}\cdot\text{CHR}'\cdot\text{CO}_2\text{Et}$  (or  $\text{Ar}\cdot\text{NH}\cdot\text{CR}\cdot\text{CR}'\cdot\text{CO}_2\text{Et}$ ), giving 4-hydroxyquinolines of type (CIII). The required arylamino-compounds have been prepared (a) from arylamines and ethyl ethoxymethylene-malonate<sup>83-88</sup> and -cyanoacetate,<sup>90</sup> ethyl formylacetate or  $\alpha$ -formylpropionate,<sup>89</sup> ethyl orthoformate and  $\text{CH}_2\text{R}'\cdot\text{CO}_2\text{Et}$  ( $\text{R}' = \text{Ph}, \text{CN}, \text{Ac}$ ),<sup>91</sup> ethyl ethoxalylacetate,<sup>96</sup> and ethyl  $\alpha$ -ethoxalylpropionate;<sup>93-95</sup> and (b) from  $\text{Ar}\cdot\text{NH}\cdot\text{COPh}$  via the imino-chloride and condensation with sodio-malonic ester.<sup>92</sup> The cyclisations in (a) were effected at *ca.* 250°; mineral oil,\* diphenyl, or Dowtherm A were used for this purpose. The following examples illustrate the scope of these syntheses: (CIII;  $\text{R} = \text{H}$ ;  $\text{R}' = \text{CO}_2\text{Et}$ ;  $\text{R}'' = \text{H}$ ,<sup>83</sup> 6- and 8- $\text{NO}_2$ ,<sup>85</sup> 6-,<sup>85</sup> 86 7-,<sup>84</sup> and 8-Cl,<sup>86</sup> 6-,<sup>84</sup> 87 7-,<sup>88</sup> and 8-OMe,<sup>88</sup> and various other substituents);<sup>85</sup> (CIII;  $\text{R} = \text{CO}_2\text{Et}$ ;  $\text{R}' = \text{Me}$ ;  $\text{R}'' = \text{Me}$ , Oalk, halogen in various positions);<sup>93, 95</sup> and (CIII;  $\text{R} = \text{CO}_2\text{Et}$ ;  $\text{R}' = \text{H}$ ;  $\text{R}'' = \text{halogen}$  in various positions).<sup>96</sup> The proportions of 5- and 7-substituted quinolines, which are to be expected when  $m\text{-C}_6\text{H}_4\text{R}''\cdot\text{NH}_2$  are used, vary, but cyclisation to a single desired position can be achieved by using 1 : 2 : 3- or 1 : 2 : 5- $\text{NH}_2\cdot\text{C}_6\text{H}_3\text{ClR}''$ , followed by catalytic dechlorination of the resultant 8-chloroquinolines.<sup>94</sup>

(2) Synthesis of 4-hydroxy-2-phenylquinolines (CIV;  $\text{R} = \text{H}$  or Me) from anthranilic acid (or its ethyl ester) and ketone acetals; the reaction,

<sup>83</sup> R. G. Gould, jun., and W. A. Jacobs, *J. Amer. Chem. Soc.*, 1939, **61**, 2890.

<sup>84</sup> C. C. Price and R. M. Roberts, *ibid.*, 1946, **68**, 1204.

<sup>85</sup> B. Riegel *et al.*, *ibid.*, p. 1264.

<sup>86</sup> D. S. Tarbell, *ibid.*, p. 1277.

<sup>87</sup> K. Schofield and J. C. E. Simpson, *J.*, 1946, 1033.

<sup>88</sup> W. M. Lauer, R. T. Arnold, B. Tiffany, and J. Tinker, *J. Amer. Chem. Soc.*, 1946, **68**, 1268.

<sup>89</sup> C. C. Price, N. J. Leonard, and R. H. Reitsema, *ibid.*, p. 1256.

<sup>90</sup> C. C. Price, N. J. Leonard, and H. F. Herbrandson, *ibid.*, p. 1251.

<sup>91</sup> H. R. Snyder and R. E. Jones, *ibid.*, p. 1253.

<sup>92</sup> R. C. Elderfield *et al.*, *ibid.*, p. 1272.

<sup>93</sup> E. A. Steck, L. L. Hallock, and A. J. Holland, *ibid.*, pp. 129, 132, 380.

<sup>94</sup> D. S. Breslow *et al.*, *ibid.*, p. 1232.

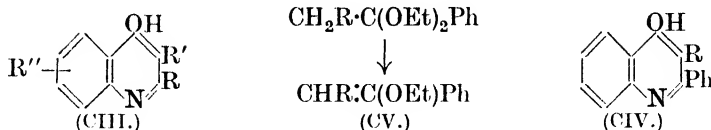
<sup>95</sup> E. A. Steck, L. L. Hallock, and A. J. Holland, *ibid.*, p. 1241.

<sup>96</sup> A. F. Surrey and H. F. Hammer, *ibid.*, pp. 113, 1244.

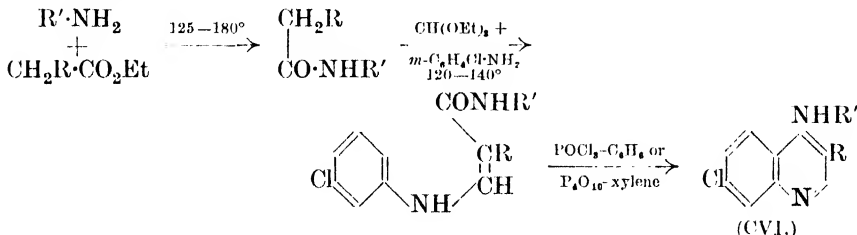
\* See also J. M. L. Stephen, I. M. Tonkin, and J. Walker, *Nature*, 1945, **156**, 629.



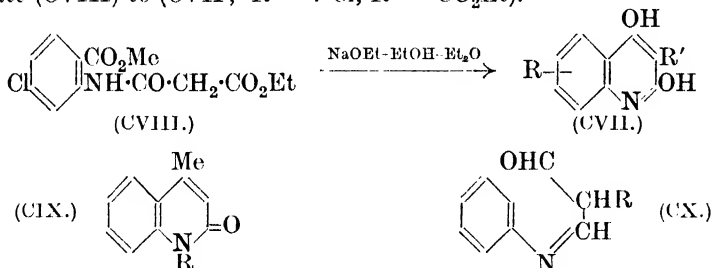
effected in diphenyl ether, is believed to proceed *via* the ethoxystyrene (CV).<sup>97</sup>



(3) Synthesis of 4-alkyl- and -aryl-aminoquinolines (CVI; R = CN and CO<sub>2</sub>Et; R' = aryl and alkyl) by the following route, which failed with aminoalkylamines.<sup>98</sup>



(4) Synthesis of 2:4-dihydroxyquinolines (a) from Ar·NH<sub>2</sub> and substituted malonic esters (in diphenyl ether), yielding (CVII; R = H, 6-OMe, 6-NMe<sub>2</sub>; R' = cyclohexyl, 3-cyclohexylpropyl)<sup>99</sup> [acetylmalonic ester (in nitrobenzene) gives 3-acetyl derivatives (as CVII; R' = Ac)<sup>100</sup>]; and (b) by low-temperature cyclisation of methyl 4-chloro-2-carbethoxyacetamidobenzoate (CVIII) to (CVII; R = 7-Cl, R' = CO<sub>2</sub>Et).<sup>101</sup>



K. N. Campbell and I. J. Schaffner<sup>102</sup> prepare lepidines from arylamines and methyl vinyl ketone or methyl 2-methoxyethyl ketone (or its acetal); an oxidising agent (FeCl<sub>3</sub>) and a condensing agent (ZnCl<sub>2</sub>, P<sub>4</sub>O<sub>10</sub>, BF<sub>3</sub>) are necessary. Acetoacetanilides, and thence substituted lepidines<sup>103</sup> and 4-methyl-1-alkyl-2-quinolones (CIX; R = alkyl),<sup>104</sup> are conveniently pre-

<sup>97</sup> R. C. Fuson and D. M. Burness, *J. Amer. Chem. Soc.*, 1946, **68**, 1270.

<sup>98</sup> C. C. Price and V. Boekelheide, *ibid.*, p. 1246.

<sup>99</sup> R. H. Baker, G. R. Lappin, and B. Riegel, *ibid.*, p. 1284.

<sup>100</sup> W. R. Vaughan, *ibid.*, p. 324.

<sup>101</sup> R. E. Lutz *et al.*, *ibid.*, p. 1285.

<sup>102</sup> *Ibid.*, 1945, **67**, 86.

<sup>103</sup> C. E. Kaslow and N. B. Sommer, *ibid.*, 1946, **68**, 644; A. L. Searles and H. G. Lindwall, *ibid.*, p. 988.

<sup>104</sup> C. E. Kaslow and D. J. Cook, *ibid.*, 1945, **67**, 1969.

pared from the amines and keten dimer; the nitration and condensation with ethyl oxalate of (CIX; R = Me) have been studied.<sup>104</sup> Renewed attention has been paid to styrylquinoline derivatives.<sup>105</sup> The Meisenheimer<sup>106</sup> preparation of 2- and 4-chloroquinoline from the oxide has been extended to *Bz*-substituted derivatives.<sup>107</sup> F. C. Uhle and W. A. Jacobs<sup>108</sup> report the synthesis of 3-nitro- and -cyano-quinolines by ring-closure of (CX; R = NO<sub>2</sub> or CN) (from arylamines and nitro- or cyano-malondialdehyde), and various 3-aminoquinolines are described by G. R. Cleino and G. A. Swan.<sup>109</sup> 7 : 8-Diaminoquinoline<sup>110</sup> and the 5 : 6-isomer<sup>111</sup> are now fairly accessible. Nitration of 4-chloroquinoline gives the 8-nitro-derivative,<sup>112</sup> whereas 4-chloroquinaldine yields 8- (mainly) with a little 5- and 6-nitro-derivative; <sup>113</sup> 4-hydroxyquinaldine yields, according to conditions, 3- and 6-nitro-4-hydroxyquinaldine.<sup>113</sup> In contrast to earlier statements, nitration of 2-chloroquinoline occurs mainly in the 8-, and only very slightly in the 5-position; the 5-nitro-derivative is best prepared from 5-nitroquinoline methiodide.<sup>114</sup> The best method for the decarboxylation of *Bz*-nitro-4-hydroxyquinoline-3-carboxylic acids appears to be pyrolysis of the silver salts.<sup>115</sup> Treatment of 8-nitro-6-methoxyquinoline with sulphuryl chloride leads to *Bz*- and *Py*-chlorination.<sup>116</sup>

### *Cinnolines.*

Some progress has been made in this field,\* which until recently was virtually unexplored. Satisfactory routes have been worked out for the preparation of 4-aryl-,<sup>117-119</sup> 4-methyl-,<sup>120-121</sup> and 4-hydroxy-cinnol-

<sup>105</sup> W. Borsche, W. Doeller, and M. Wagner-Roemmich, *Ber.*, 1943, **76**, 1099; V. A. Petrov, *J.*, 1945, 18; R. S. Tipson, *J. Amer. Chem. Soc.*, 1945, **67**, 507; M. A. Clapp and R. S. Tipson, *ibid.*, 1946, **68**, 1332; M. V. Rubtsov and V. J. Bunina, *J. Gen. Chem. Russia*, 1944, **14**, 1128.

<sup>106</sup> J. Meisenheimer, *Ber.*, 1926, **59**, 1848; B. Bobranski, *ibid.*, 1938, **71**, 578.

<sup>107</sup> G. B. Bachman and D. E. Cooper, *J. Org. Chem.*, 1944, **9**, 302; see also, *e.g.*, H. Gilman and S. M. Spatz, *J. Amer. Chem. Soc.*, 1944, **66**, 621; A. G. Renfrew, *ibid.*, 1946, **68**, 1433.

<sup>108</sup> *J. Org. Chem.*, 1945, **10**, 76.

<sup>109</sup> *J.*, 1945, 867.

<sup>110</sup> F. Linsker and R. L. Evans, *J. Amer. Chem. Soc.*, 1946, **68**, 149.

<sup>111</sup> *Idem*, *ibid.*, p. 874; D. M. Hall and E. E. Turner, *J.*, 1945, 699.

<sup>112</sup> R. H. Baker, C. J. Albisetti, jun., R. M. Dodson, G. R. Lappin, and B. Riegel, *J. Amer. Chem. Soc.*, 1946, **68**, 1532.

<sup>113</sup> B. E. Halcrow and W. O. Kermack, *J.*, 1945, 415.

<sup>114</sup> A. J. Deinet and R. E. Lutz, *J. Amer. Chem. Soc.*, 1946, **68**, 1325.

<sup>115</sup> R. H. Baker, G. R. Lappin, C. J. Albisetti, jun., and B. Riegel, *ibid.*, p. 1267.

<sup>116</sup> J. Schultz, M. A. Goldberg, G. Carsch, and E. P. Ordas, *J. Org. Chem.*, 1946, **11**, 170.

<sup>117</sup> R. Stoermer and H. Fincke, *Ber.*, 1909, **42**, 3115; R. Stoermer and O. Gaus, *ibid.*, 1912, **45**, 3104; J. C. E. Simpson and O. Stephenson, *J.*, 1942, 353.

<sup>118</sup> J. C. E. Simpson, *J.*, 1943, 447.

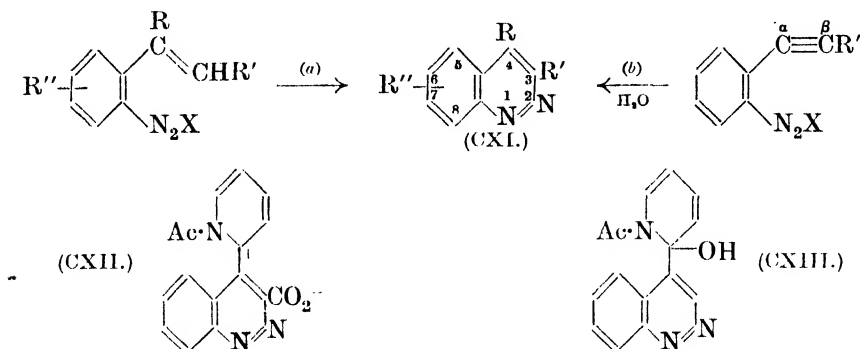
<sup>119</sup> J. C. E. Simpson, *J.*, 1946, 673.

<sup>120</sup> T. L. Jacobs, S. Winstein, R. B. Henderson, and E. C. Spaeth, *J. Amer. Chem. Soc.*, 1946, **68**, 1310.

<sup>121</sup> C. M. Atkinson and J. C. E. Simpson, in the press.

\* For a review of the early literature see N. J. Leonard, *Chem. Rev.*, 1945, **37**, 269.

ines<sup>122-124</sup> (as CXI). The essential reaction is the intramolecular cyclisation of a diazotised *o*-aminoaryl-ethylene or -acetylene,<sup>122</sup> as indicated in the accompanying scheme. Reaction (a) occurs (i) when R' = alkyl, aryl,



or aralkyl, R being alkyl or aryl, but not hydrogen; <sup>118, 119</sup> (ii) when R = OH and R' = hydrogen or methyl (*i.e.*, diazotised *o*-amino-aceto- and -propio-phenones), these cyclisations being favoured by electron-attractive substituents (R'') in suitable positions, *e.g.*, 6- and 8-Cl, -NO<sub>2</sub>, etc. (cinnoline numbering), although at very low pH and moderate temperatures the reactions proceed smoothly even when R'' is hydrogen.<sup>125</sup> Reaction (b), leading to 4-hydroxy-derivatives, occurs when R' = H or CO<sub>2</sub>H, and in both (a) and (b) the necessary criterion is the development of sufficient anionoid activity on C<sub>β</sub> to result in co-ordination with the kationoid diazonium grouping.<sup>122</sup> The basic centre in 4-methylcinnolines is N<sub>1</sub>,<sup>121</sup> and the 4-methyl group is reactive.<sup>120, 121</sup> The presence in the cinnoline molecule of the second nitrogen atom may profoundly affect the fine structure, and hence the reactivity, of the nucleus as compared with corresponding quinoline derivatives; the most striking example of this which has yet emerged is to be seen in the action of pyridine and acetic anhydride on 4-hydroxy-cinnoline- and -quinoline-3-carboxylic acid; the latter is unchanged, whereas the cinnoline derivative combines with both reagents, yielding (CXII) and (CXIII), which in turn undergo various further transformations.<sup>126</sup>

### *Pterins.*

Interest has been sharply focused on this group of natural products, which are derivatives of the hypothetical "pteridine" (CXIV),<sup>127</sup> by the synthesis of the liver *L. casei* factor (CXLV),<sup>171, 174</sup> in which the ring-

<sup>122</sup> K. Schofield and J. C. E. Simpson, *J.*, 1945, 512, 520.

<sup>123</sup> N. J. Leonard and S. N. Boyd, jun., *J. Org. Chem.*, 1946, **11**, 419.

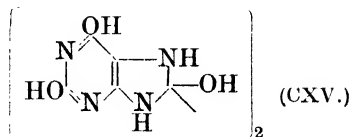
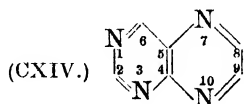
<sup>124</sup> C. M. Atkinson and J. C. E. Simpson, *J.*, 1947, 232; J. R. Keneford and J. C. E. Simpson, *ibid.*, p. 227.

<sup>125</sup> J. R. Keneford and J. C. E. Simpson, in the press.

<sup>126</sup> K. Schofield and J. C. E. Simpson, *J.*, 1946, 472.

<sup>127</sup> C. Schöpf and R. Reichert, *Annalen*, 1941, **548**, 82.

system (CXIV) is incorporated, and by the discovery <sup>128, 129</sup> that several members of this group are biologically active.



The naturally-occurring pterins are mostly colourless or yellow compounds which show marked fluorescence in solution at or near pH 7. They are found in the wings of various species of butterflies and wasps,<sup>130-133</sup> in the skin<sup>134</sup> and eyes<sup>135</sup> of fishes, and in the urine and liver of mammals.<sup>136-138</sup> At an early stage of the researches on these compounds, which were carried out in the laboratories of H. Wieland and C. Schöpf, it was recognised<sup>131</sup> that a close relationship exists between the purines and leucopterin, one of the commonly-occurring pterins, and structure (CXV) was proposed<sup>131</sup> for this substance. Soon afterwards,<sup>132</sup> it became necessary to discard the formula  $C_{10}H_{10}O_6N_8$  in view of the isolation of guanidine as a hydrolytic product of a leucopterin derivative, and the loss of  $\frac{1}{3}$  of the total nitrogen of leucopterin on treatment with nitrous acid; to reconcile these facts with the analytical data, a  $C_{19}N_{15}$  formulation was adopted, and this persisted, with minor variations, for some years. During this period the chemistry of leucopterin and of its analogue, xanthopterin, was painstakingly developed with small quantities of material; and, although it was noticeable that many of the reactions (acetylation, chlorination with phosphorus pentachloride, action of nitrous acid, formation of glycol derivatives on oxidation with chlorine in various media) occurred in triplicate (*i.e.*, implied the presence of three similarly-reacting groups in the pterin molecules), simplified molecular formulæ were not warranted in face of the analytical data. The practical difficulties of the problem were unusually formidable. Apart from the labour of the isolation of pterins, which involved the collection and manipulation of many thousands of butterflies of a given species, these substances are insoluble in organic solvents, are difficult to crystallise and purify, and decompose without melting; they occur as hydrates which retain water very tenaciously and give spurious analytical data under ordinary conditions.

<sup>128</sup> R. Tschesche and H. J. Wolf, *Z. physiol. Chem.*, 1937, **248**, 34.

<sup>129</sup> M. Polonovski, R.-G. Busnel, and M. Pesson, *Helv. Chim. Acta*, 1946, **29**, 1328.

<sup>130</sup> H. Wieland and C. Schöpf, *Ber.*, 1925, **58**, 2178.

<sup>131</sup> C. Schöpf and H. Wieland, *ibid.*, 1926, **59**, 2067.

<sup>132</sup> H. Wieland, H. Metzger, C. Schöpf, and M. Bülow, *Annalen*, 1933, **507**, 226.

<sup>133</sup> C. Schöpf and E. Becker, *ibid.*, p. 266; 1936, **524**, 49; E. Becker and C. Schöpf, *ibid.*, p. 124.

<sup>134</sup> R. Hüttel and G. Sprengling, *ibid.*, 1943, **554**, 69; M. Polonovski, R.-G. Busnel, and M. Pesson, *Compt. rend.*, 1943, **217**, 163.

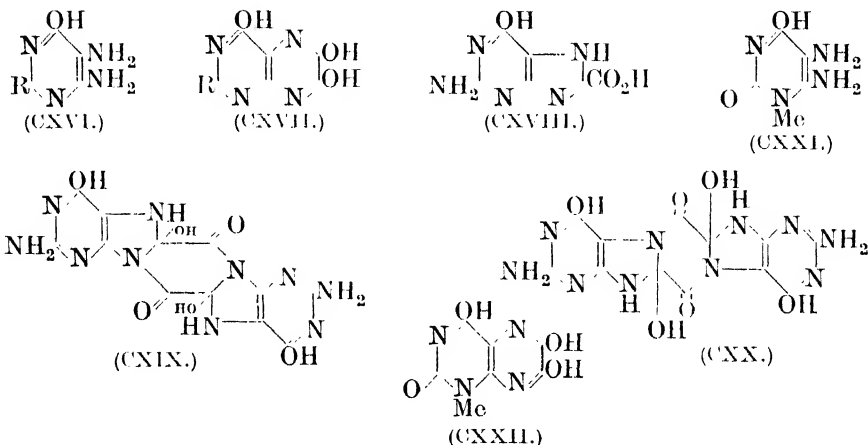
<sup>135</sup> A. Pirie and D. M. Simpson, *Biochem. J.*, 1946, **40**, 14.

<sup>136</sup> W. Koschura, *Z. physiol. Chem.*, 1936, **240**, 127.

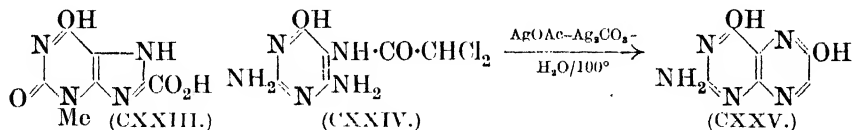
<sup>137</sup> *Idem*, *ibid.*, 1943, **277**, 159.

<sup>138</sup> *Idem*, *ibid.*, p. 284; **279**, 44.

In 1940 the analytical difficulties were recognised and largely overcome,<sup>139</sup> and the formula of leucopterin was disclosed as  $(C_6H_5O_3N_5)_x$ , where  $x = 1, 2$ , or  $3$ . Synthesis of the pterin by fusion of 2:4:5-triamino-6-hydroxypyrimidine (CXVI;  $R = NH_2$ ) with oxalic acid<sup>140</sup> restricted the possible structures to (CXVII;  $R = NH_2$ ), (CXVIII), (CXIX), and (CXX). Of these (CXVIII) was excluded by the results of further applications of the oxalic acid synthesis. Condensation of 4:5-diamino-2:6-



dihydroxypyrimidine (CXVI;  $R = OH$ ) and of 4:5-diamino-6-hydroxy-3-methyl-2-pyrimidone (CXXI) with oxalic acid at about  $250^\circ$ <sup>141</sup> gave, respectively, 2:6:8:9-tetrahydroxypteridine ("deaminoleucopterin") (CXVII;  $R = OH$ ) and the 3-methyl analogue (CXXII), which was not identical with 3-methylxanthine-8-carboxylic acid (CXXIII),<sup>142</sup> thus excluding the alternative ring-closure of (CXXI). Structure (CXVIII) for leucopterin is thus eliminated if (as is very probable) the oxalic acid condensations to give leucopterin, deaminoleucopterin, and (CXXII) all pro-



ceed in the same sense; and this was proved for leucopterin and deaminoleucopterin by the conversion of the former into the latter by means of nitrous acid.<sup>132</sup> Further weight is given to this argument by the synthesis of 6-deoxyleucopterin (CXXIX; see below), a transformation product of leucopterin, by the oxalic acid method. Furthermore, purine-8-carboxylic acids are readily decarboxylated, whereas leucopterin does not decompose below  $400^\circ$ .

<sup>139</sup> H. Wieland and R. Purrmann, *Annalen*, 1940, **544**, 163.

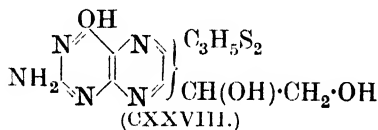
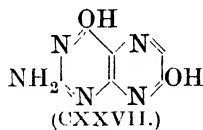
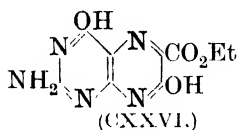
<sup>140</sup> R. Purrmann, *ibid.*, p. 182.

<sup>141</sup> *Idem*, *ibid.*, 1941, **546**, 98.

<sup>142</sup> W. Traube, *ibid.*, 1923, **432**, 266.

The choice between (CXVII;  $R = NH_2$ ), (CXIX), and (CXX) as the structure of leucopterin was finally settled by the elucidation of the constitution of xanthopterin, another naturally-occurring pterin, and of its relationship to leucopterin. Fusion of (CXVI;  $R = NH_2$ ) with dichloroacetic \* acid gave the amide (CXXIV), which on cyclisation under mild conditions yielded xanthopterin; <sup>141</sup> this is therefore 2-amino-6 : 8-dihydroxy-pteridine (CXXV). Now xanthopterin takes up an atom of oxygen in contact with platinum in weakly acid solution, yielding leucopterin <sup>139</sup> (the same result is obtained by treatment of xanthopterin with methylene-blue and an enzyme preparation,<sup>145</sup> and by the prolonged action of hydrogen peroxide),<sup>143</sup> and, as leucopterin is devoid of peroxidic properties,<sup>143</sup> the reaction can only be represented by hydroxylation of xanthopterin at  $C_9$ . Leucopterin is therefore (CXVII;  $R = NH_2$ ).

A third naturally-occurring pterin is 8-deoxyleucopterin or *isoxanthopterin* (CXXVII), which was synthesised by hydrolysis, followed by decarboxylation, of the ester (CXXVI), obtained from (CXVI;  $R = NH_2$ ) and diethyl ketomalonate.<sup>144</sup> When this pterin was first isolated,<sup>132</sup> it was



given the name of anhydroleucopterin; the above synthesis, however, discloses its relationship to leucopterin, and it has been obtained from leucopterin by electrolytic reduction.<sup>145</sup> The reverse reaction, *viz.*, oxidation of *isoxanthopterin* to leucopterin, has not yet been achieved, but the action on *isoxanthopterin* of nitrous acid and of chlorine water gives the leucopterin derivatives (CXVII;  $R = OH$ ) and (CXXXII;  $R = H$ ) respectively.<sup>146</sup>

Several other † natural pterins have been described. Uropterin, isolated from urine,<sup>136</sup> has been proved to be xanthopterin.<sup>137</sup> Another urinary pterin, urothion,<sup>138</sup> is more complex. Its molecular formula is  $C_{11}H_{13}O_3N_5S_2$ . Both sulphur atoms are inert, and no thiol group is present. The pigment, unlike other pterins, is optically active. Although there is as yet no rigid

<sup>143</sup> H. Wieland and R. Purrmann, *Annalen*, 1939, **539**, 179.

<sup>144</sup> R. Purrmann, *ibid.*, 1941, **548**, 284.

<sup>145</sup> H. Wieland and R. Liebig, *ibid.*, 1944, **555**, 146.

<sup>146</sup> H. Wieland, A. Tartter, and R. Purrmann, *ibid.*, 1940, **545**, 209.

\* The use of the bisulphite compound of barium glyoxylate in sulphuric acid, instead of dichloroacetic acid, gives a greatly improved yield.<sup>137</sup>

† An interesting general account of the occurrence of pterins in wing-pigments is given by (Sir) F. G. Hopkins (*Proc. Roy. Soc.*, 1942, *B*, **130**, 359). It should be noted that the purple pigment, rhodopterin, which is there discussed, is not a true pterin, but a condensation product of leucopterin and xanthopterin-9-carboxylic acid, and that it has been re-named pterorhodin (R. Purrmann and M. Maas, *Annalen*, 1944, **556**, 186).

proof that the molecule of urothion contains the pteridine skeleton, the expression (CXXXVIII) has been advanced on the basis of the foregoing data and the following evidence. Urothion yields a tetra-acetyl derivative, which gives satisfactory cryoscopic molecular weight values and can be hydrolysed to a monoacetyl derivative. The pigment is amphoteric, and amino-nitrogen estimations suggest the presence of a guanidine residue; its ultra-violet absorption spectrum resembles those of xanthopterin, riboflavin, and other *isoalloxazines*; and periodic acid oxidation yields formaldehyde and a product,  $C_{10}H_9O_2N_5S_2$  (urothionaldehyde). The pigment also gives, with concentrated sulphuric acid, the red colour (thiophenol reaction) characteristic of compounds containing a thiol, or potential thiol, group attached to an aromatic ring.

The fish-skin pigment, ichthyopterin<sup>134</sup> (also known as fluoresceyanine),<sup>129</sup> is likewise a pterin of unknown structure. It is reduced by fuming hydriodic acid with liberation of iodine, and on dilution the leuco-compound is reoxidised by the iodine. This extremely easy *reversible* oxidation-reduction is shown only by *isoxanthopterin*<sup>146</sup> and the acid<sup>144</sup> obtained by hydrolysis of (CXXXVI). Xanthopterin is also reduced under the same conditions,<sup>146</sup> but the dihydro-compound is not reoxidised by iodine, although it can be oxidised to the pterin by a variety of other reagents.<sup>147</sup> Leucopterin, on the other hand, is much more difficult to reduce,<sup>145, 146</sup> but under appropriate conditions yields *isoxanthopterin*<sup>145</sup> or dihydroxanthopterin.<sup>147</sup> \* A controlling factor affecting the redox potential thus appears to be the point of hydroxylation of the pyrazine ring; for this reason, and also because the absorption spectra of *isoxanthopterin* and *ichthyopterin* are very similar, it has been suggested<sup>134</sup> that this marine pterin is a derivative of 9-hydroxy-pteridine. It is to be noted, however, that the suggested molecular formula,  $C_7H_8O_3N_4$ , implies that it is a dihydro-derivative of this ring-system.

*Properties of Pterins.*—Excluding differences in elementary composition, the criteria by which individual pterins can most readily be distinguished are basicity (this may be very slight or considerable; acidic properties are well-marked), fluorescence and the effect of pH thereon, absorption spectra, and the redox reaction already noted. The fluorescence of pterins has been studied in some detail; <sup>134, 148, 149, 150</sup> leucopterin exhibits its maximum fluorescence in strongly alkaline solution,<sup>149</sup> but under these conditions xanthopterin fluoresces only slightly, the intensity increasing rapidly with fall in pH.<sup>149</sup> Various measurements of the ultra-violet absorption spectra of pterins have been recorded (frequently with similar data for purines and

<sup>147</sup> J. R. Totter, *J. Biol. Chem.*, 1944, **154**, 105.

<sup>148</sup> W. Jacobson and D. M. Simpson, *Biochem. J.*, 1946, **40**, 3, 9.

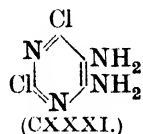
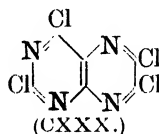
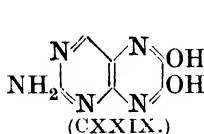
<sup>149</sup> P. Decker, *Z. physiol. Chem.*, 1942, **274**, 223.

<sup>150</sup> M. Polonovski, S. Guinand, M. Pesson, and R. Vieillefosse, *Bull. Soc. chim.*, 1945, **12**, 924.

\* The reduction of leucopterin to dihydroxanthopterin, followed by oxidation of the latter with alkaline silver nitrate, enables a convenient synthesis of xanthopterin to be effected from (CXVI;  $R = NH_2$ ) and oxalic acid.<sup>147</sup>

flavins), but no systematic study has yet been made.<sup>136, 138, 150-152, 162</sup> A useful, but not exhaustive, summary of the chemical and optical data is given by W. Jacobson and D. M. Simpson.<sup>148</sup>

When leucopterin is treated with phosphorus pentachloride a mono-chloro-derivative is obtained. That the 6-position is involved in this reaction was shown by reduction of the chloro-compound to the deoxy-derivative (CXXIX), which was synthesised from 2 : 4 : 5-triaminopyrimidine and oxalic acid.<sup>146</sup> Application of the reaction to deaminoleucopterin (CXVII; R = OH) gave, similarly, the 2 : 6-dichloro-compound,<sup>153</sup> but under different conditions of isolation 2 : 6 : 8 : 9-tetrachloropteridine (CXXX)



was obtained, and it was found that the 2 : 6-dichloro-derivative had been formed \* by partial hydrolysis of (CXXX) under the conditions of isolation. In contrast, drastic alkaline hydrolysis was needed to convert the dichloro-derivative (2 : 6-dichloro-8 : 9-dihydroxypteridine) into (CXVII; R = OH); the corresponding dichloropyrimidine (CXXXI) is also resistant.<sup>153</sup> Incidentally it may be noted that the production of a tetrachloro-derivative from deaminoleucopterin is not possible on the basis of the purine-8-carboxylic acid structure (CXVIII) for leucopterin; its formation thus constitutes an independent proof of the correctness of (CXVII; R = NH<sub>2</sub>).

Before the constitution of leucopterin and xanthopterin had been settled by synthesis, a number of degradation products had been isolated during attempts to unravel the complexities of the supposed C<sub>19</sub> structures. Formulation of these reactions on the basis of (CXIV) illustrates clearly the close parallel between them and well-known purine transformations. Oxidation of leucopterin with chlorine water or chlorine in methanol leads to the glycol (CXXXII; R = H) or its dimethyl ether (CXXXII; R = Me) respectively.<sup>132</sup> Hydrolysis of (CXXXII; R = H) results, as with uric acid, in the conversion of the pyrimidine into a hydantoin ring and in the isolation of derivatives of the latter, *viz.*, (CXXXIII), (CXXXIV), and (CXXXV).<sup>139, 154</sup> The product formed by the action of chlorine in acetic acid on leucopterin is (CXXXVI),<sup>132, 139</sup> corresponding to the formation of

<sup>151</sup> H. K. Mitchell, *J. Amer. Chem. Soc.*, 1944, **66**, 274.

<sup>152</sup> H. Fromherz and A. Kotzschmar, *Annalen*, 1938, **534**, 283.

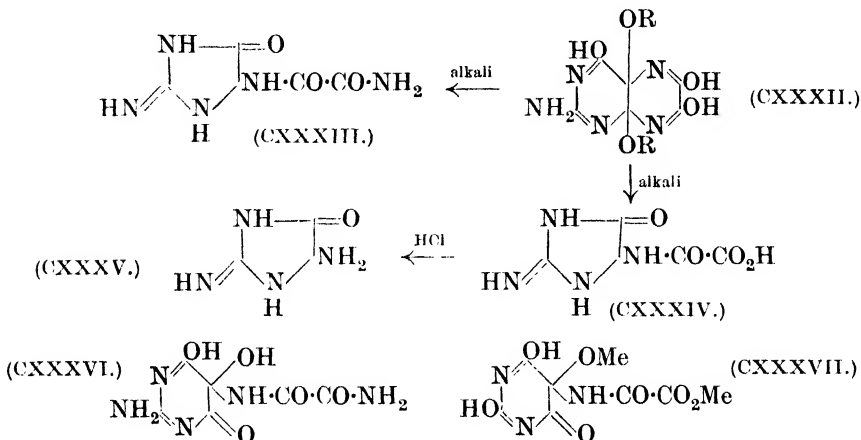
<sup>153</sup> C. Schöpf and R. Reichert, *ibid.*, 1941, **548**, 82.

<sup>154</sup> H. Wieland and A. Kotzschmar, *ibid.*, 1937, **530**, 152.

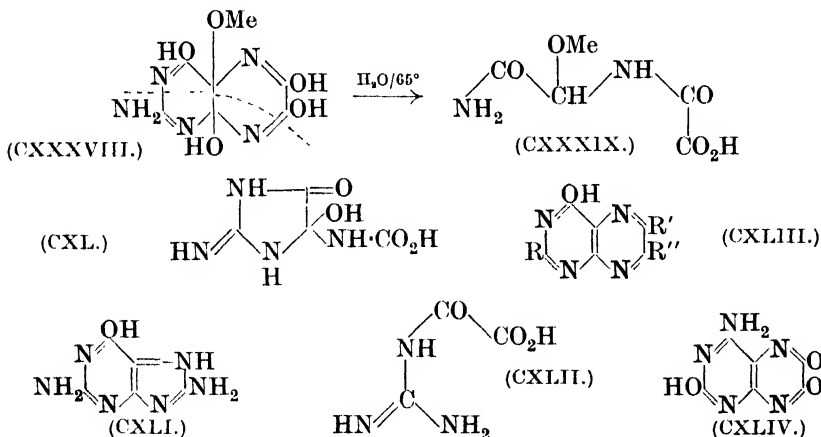
\* It is of considerable interest that (CXXX) is apparently more readily hydrolysed in alkaline than in acid solution; this is in direct contrast to recent evidence (see, for example, C. K. Banks, *J. Amer. Chem. Soc.*, 1944, **66**, 1127, 1131; A. J. Tomisek and B. E. Christensen, *ibid.*, 1945, **67**, 2112; C. K. Banks and J. Controulis, *ibid.*, 1946, **68**, 944) that hydrolysis of chloro-heterocyclic compounds is acid-catalysed, and suggests that a different mechanism may be operative in the hydrolysis of (CXXX).



5-hydroxypseudouric acid from uric acid;<sup>155</sup> analogously, oxidation of deaminoleucopterin (CXXVII; R = OH) with chlorine in methanol gives



(CXXXVII).<sup>156</sup> The glycol ether (CXXXII; R = Me) is extremely unstable; in aqueous solution at 40° it yields the monoether (CXXXVIII), which readily decomposes further into (CXXXIX), guanidine, and carbon dioxide by hydrolytic fission.<sup>132</sup> Reference has already been made to the formation of leucopterin by hydrogen peroxide oxidation of xanthopterin;



the reaction is not, however, quantitative, and under suitable conditions imino-oxonic acid (CXL) is also formed.<sup>139</sup> This reaction resembles the oxidation of uric to oxonic acid,<sup>157</sup> and, indeed, (CXL) is also formed from

<sup>155</sup> H. Biltz and M. Heyn, *Annalen*, 1917, **413**, 7.

<sup>156</sup> H. Wieland and A. Tartter, *ibid.*, 1940, **543**, 287.

<sup>157</sup> F. J. Moore and R. M. Thomas, *J. Amer. Chem. Soc.*, 1918, **40**, 1120; H. Biltz and R. Robl, *Ber.*, 1920, **53**, 1967.

the purine (CXLI).<sup>139</sup> Oxidation of xanthopterin with hot sodium chlorate and acid, or with cold nitrosylsulphuric acid, brings about disruption of the pyrimidine as well as of the pyrazine ring, and oxalylguanidine (CXLII) is formed; this is also produced by similar treatment of (CXVI;  $R = NH_2$ ).<sup>158</sup>

*Other Synthetic Pterins.*—M. Polonovski, R. Vieillefosse, and M. Person<sup>159</sup> have prepared, from (CXVI;  $R = SH$ ) and 1:2-dicarbonyl compounds, three non-fluorescent 2-mercaptopterins (CXLIII;  $R = SH$ ;  $R' = H$ ,  $CO_2H$ ,  $Ph$ ;  $R'' = H$ ,  $OH$ ,  $Ph$ ), which were converted into 2-hydroxy-analogues<sup>160, 161</sup> by alkaline hydrogen peroxide; these authors have also shown that the mercaptopterins undergo *S*-ethylation, and they conclude, from the fluorescence shown by the *S*-alkyl- and the hydroxy- (in contrast to the 2-thiol) derivatives, that the characteristic fluorescence of pterins depends on the preservation of an intact \* aromatic structure in the pyrimidine ring, *i.e.*, the 2-hydroxy-compounds exist as such whereas the 2-thiol derivatives exist in the tautomeric form. Condensations between (CXVI;  $R = NH_2$  and  $OH$ ) and  $\alpha$ -diketones have been extended to include phenanthraquinone and acenaphthenequinone.<sup>162</sup> The original use<sup>132</sup> of the term “*isoleuco-pterin*” now appears unwarranted;<sup>146</sup> instead, the name is given to the synthetic pterin (CXLIV).<sup>145</sup> This substance, unlike leucopterin, fails to react with nitrous acid (*isoguanine* and *guanine* are similarly differentiated);<sup>145</sup> on the other hand, the xanthopterin molecule is disrupted by this reagent and does not yield the deaminoxanthopterin (CXLIII;  $R = R' = OH$ ;  $R'' = H$ ) obtainable from (CXVI;  $R = OH$ ) and the bisulphite compound of glyoxylic acid.<sup>145, 158</sup>

*The Vitamin B<sub>6</sub> Problem.*†—Casual observation of progress in this field has hitherto been somewhat difficult owing to the apparent complexity of the problem. At an early stage in the purification of the one or more growth factors having antianæmic and/or microbiological (*L. casei*  $\epsilon$ , *S. lactis* R, *S. faecalis* R) growth-stimulating properties it became clear that the biological characteristics of the product were dependent on the source (liver, yeast, spinach, and other vegetable sources). Thus Peterson and

<sup>158</sup> C. Schöpf and A. Kottler, *Annalen*, 1939, **539**, 128.

<sup>159</sup> *Bull. Soc. chim.*, 1945, **12**, 78; see also ref. 150.

<sup>160</sup> R. Kuhn and A. H. Cook, *Ber.*, 1937, **70**, 761.

<sup>161</sup> K. Ganapati, *J. Indian Chem. Soc.*, 1937, **14**, 627.

<sup>162</sup> C. K. Kain, M. F. Mallette, and E. C. Taylor, jun., *J. Amer. Chem. Soc.*, 1946, **68**, 1996.

\* In the opinion of the Reporter, a direct correlation of fluorescence with “aromaticity” produced *via* prototropy seems to be an over-simplification. Arguments which may have some bearing on this point, and which are certainly relevant to the whole question of the fine structure of pterins and other hydroxy-heterocyclic nitrogen compounds, have recently been advanced by F. Arndt (*Rev. Fac. Sci. Univ. Istanbul*, 1944, **9**, 19), who discusses the conception that the “aromaticity” of such compounds is compatible with their existence in the keto-dihydro- (CO-NH) form by virtue of an electromeric shift to  $\bar{O}-C^+=NH$ , and is thus potentially independent of tautomerism.

† This problem is dealt with later in its biochemical aspect (p. 296).

his co-workers<sup>163</sup> obtained from liver and from yeast a "norite eluate factor" essential for growth of *L. casei* (*L. helveticus*), which also appeared to be vital to the growth of chicks;<sup>164</sup> and the preparation from spinach of a factor, designated folic acid, having growth-stimulating properties for *L. casei*, *S. lactis* R, and other bacteria, was reported almost simultaneously by H. K. Mitchell *et al.*<sup>165</sup> Later, J. J. Piffner *et al.*<sup>166</sup> described the isolation of a crystalline antianæmic factor from liver extracts, which, following an earlier suggestion,<sup>167</sup> was named vitamin B<sub>c</sub>; identity of this substance with the norite eluate factor was claimed,<sup>166</sup> and identity with folic acid was suggested.<sup>166</sup> It was then found by J. C. Keresztesy *et al.*<sup>168</sup> that "various types of extracts and liver preparations" yielded a substance which, although much more active than folic acid for *S. lactis* R, was inactive for *L. casei*, whereas folic acid is equally effective for each micro-organism. On the other hand, E. L. R. Stokstad, working with crystalline preparations from liver and from yeast,<sup>169</sup> found that the liver factor was equally active for *L. casei* and for *S. lactis* R, but that the yeast factor was only half as active as the liver preparation for *S. lactis* R, whereas both preparations were equally effective for *L. casei*; and a new *L. casei* factor from an undisclosed source<sup>170</sup> (later described<sup>171</sup> as a fermentation residue; the factor is named the fermentation *L. casei* factor)<sup>171, 174</sup> was stated to be 85—90% as active as the norite eluate (liver) factor for *L. casei*, but only 6% as active for *S. lactis* R.

It is clear from these results that several factors are involved, and this conclusion is substantiated by the results of antianæmic studies. Following the original observation that monkey anæmia could be cured by yeast extracts ("vitamin M"),<sup>172</sup> it was found that chicken anæmia could likewise be cured by a crystalline yeast factor and also by vitamin B<sub>c</sub>, which was chemically distinct from the yeast factor.<sup>173</sup> Vitamin B<sub>c</sub> thus possesses

<sup>163</sup> E. E. Snell and W. H. Peterson, *J. Bact.*, 1940, **39**, 273; B. L. Hutchings, N. Bohonos, and W. H. Peterson, *J. Biol. Chem.*, 1941, **141**, 521.

<sup>164</sup> B. L. Hutchings, N. Bohonos, D. M. Hegsted, C. A. Elvehjem, and W. H. Peterson, *J. Biol. Chem.*, 1941, **140**, 681.

<sup>165</sup> H. K. Mitchell, E. E. Snell, and R. J. Williams, *J. Amer. Chem. Soc.*, 1941, **63**, 2284; 1944, **66**, 267. See also E. H. Frieden, H. K. Mitchell, and R. J. Williams, *ibid.*, 1944, **66**, 269; H. K. Mitchell and R. J. Williams, *ibid.*, p. 271; H. K. Mitchell, *ibid.*, p. 274.

<sup>166</sup> J. J. Piffner, S. B. Binkley, E. S. Bloom, R. A. Brown, O. D. Bird, A. D. Emmett, A. G. Hogan, and B. L. O'Dell, *Science*, 1943, **97**, 404.

<sup>167</sup> A. G. Hogan and E. M. Parrott, *J. Biol. Chem.*, 1940, **132**, 507.

<sup>168</sup> J. C. Keresztesy, E. L. Rickes, and J. L. Stokes, *Science*, 1943, **97**, 465.

<sup>169</sup> E. L. R. Stokstad, *J. Biol. Chem.*, 1943, **149**, 573.

<sup>170</sup> B. L. Hutchings, E. L. R. Stokstad, N. Bohonos, and N. H. Slobodkin, *Science*, 1944, **99**, 371; see also E. S. Bloom, J. M. Vandenbelt, S. B. Binkley, B. L. O'Dell, and J. J. Piffner, *ibid.*, **100**, 295.

<sup>171</sup> R. B. Angier *et al.* (for names see ref. 174), *ibid.*, 1945, **102**, 227.

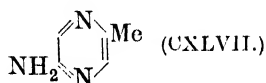
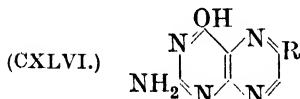
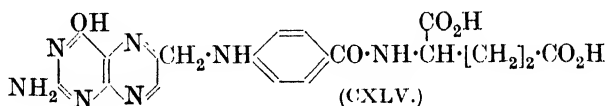
<sup>172</sup> P. L. Day, W. C. Langston, and W. J. Darby, *Proc. Soc. Exp. Biol. Med.*, 1938, **38**, 860.

<sup>173</sup> J. J. Piffner, D. G. Calkins, B. L. O'Dell, E. S. Bloom, R. A. Brown, C. J. Campbell, and O. D. Bird, *Science*, 1945, **102**, 228.

both antianæmic and microbiological (*L. casei*  $\epsilon$ ) activity; <sup>166</sup> the crystalline yeast factor (known as vitamin B<sub>6</sub> conjugate), on the other hand, has very little microbiological activity (*L. casei*, *S. faecalis*), but yields vitamin B<sub>6</sub> on enzymic digestion.<sup>173</sup>

From this seemingly confused background the following clarifications have emerged as a result of recent work : (a) proof of structure and synthesis of the liver *L. casei* factor (pteroylglutamic acid); (b) identification of pteroylglutamic acid with vitamin B<sub>6</sub>; (c) establishment of the chemical relationship between vitamin B<sub>6</sub>, vitamin B<sub>6</sub> conjugate, and the fermentation *L. casei* factor.

*Structure of L. casei Factor.*—The constitution of this substance has been proved to be (CXLV) by a group of sixteen workers in the following manner.<sup>171, 174</sup> Fission with sulphurous acid of the fermentation *L. casei* factor yielded an amine fraction (a) together with 2-amino-6-hydroxy-pteridine-8-aldehyde (CXLVI; R = CHO). The orientation of the aldehyde was determined (i) by its conversion under anærobic alkaline conditions into the corresponding acid (CXLVI; R = CO<sub>2</sub>H) and (CXLVI; R = Me), followed by vigorous hydrolysis of the latter, by the method of J. Weijlard



*et al.*,<sup>175</sup> to the known <sup>175</sup> 2-amino-5-methylpyrazine (CXLVII); (ii) by the conversion of the known acid derived from (CXXVI)<sup>144</sup> into (CXLVI; R = CO<sub>2</sub>H) by means of phosphorus pentachloride and hydriodic acid; and (iii) by decarboxylation of (CXLVI; R = CO<sub>2</sub>H) and synthesis of the resultant 8-deoxyxanthopterin from (CXVI; R = NH<sub>2</sub>) and glyoxal. The 8-methyl derivative (CXLVI; R = Me) was also obtained by decarboxylation of (CXLVI; R = CH<sub>2</sub>CO<sub>2</sub>H), itself prepared by condensation of (CXVI; R = NH<sub>2</sub>) and ethyl 2-keto-3:3-dimethoxy-*n*-butyrate. Acid hydrolysis of the amine fraction (a) gave *p*-aminobenzoic acid and glutamic acid (3 mols.).

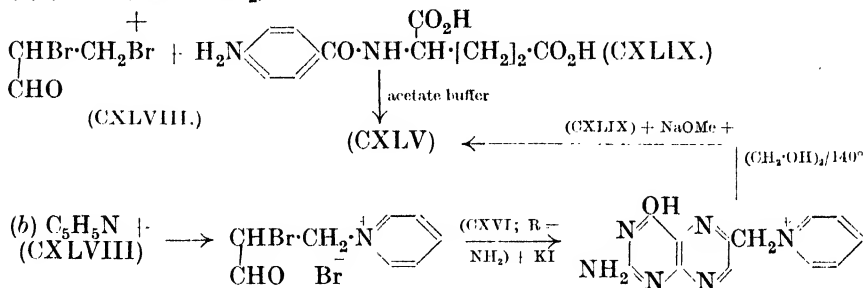
The fermentation *L. casei* factor was converted by anærobic alkaline hydrolysis into the liver *L. casei* factor and an  $\alpha$ -amino-acid (2 mols.); aerobic alkaline hydrolysis, on the other hand, gave (CXLVI; R = CO<sub>2</sub>H) and an amine fraction from which *p*-aminobenzoic acid was obtained by further

<sup>174</sup> R. B. Angier, J. H. Booth, B. L. Hutchings, J. H. Mowat, J. Semb, E. L. R. Stokstad, Y. SubbaRow, C. W. Waller, D. B. Cosulich, M. J. Fahrenbach, M. E. Hultquist, E. Kuh, E. H. Northey, D. R. Seeger, J. P. Sickels, and J. M. Smith, jun., *ibid.*, 1946, 103, 667.

<sup>175</sup> J. Weijlard, M. Tishler, and A. E. Erickson, *J. Amer. Chem. Soc.*, 1945, 67, 802.

hydrolysis. Consideration of these results, together with those obtained by the sulphurous acid degradation, indicated that the liver *L. casei* factor has the structure (CXLV), and that the introduction of two more glutamic acid residues into this molecule produces the fermentation *L. casei* factor. Synthesis of the liver *L. casei* factor was achieved by two methods as shown in the accompanying scheme, the yield in each case being *ca.* 15%. The intermediate (CXLIX) was derived from *l*(+)-glutamic acid. It will be noted that each synthesis proceeds through a dihydro-derivative and subsequent *in situ* oxidation.

(a) (CXVI; R = NH<sub>2</sub>)



J. J. Piffner *et al.* have shown<sup>176</sup> that vitamin B<sub>6</sub> conjugate consists of the vitamin combined with six glutamic acid residues in peptide form; comparison of the vitamin itself with pteroylglutamic acid showed that the two substances are identical. The biological specificity of vitamin B<sub>6</sub> conjugate, of pteroylglutamic acid, and of the fermentation *L. casei* factor thus depends on the nature of the acid side chain (or chains) attached to a common nucleus; and this conception is strengthened by the observation of Angier *et al.*<sup>174</sup> that, if *p*-aminobenzoic acid is used instead of (CXLIX), syntheses (a) and (b) lead to a product which is active for *S. faecalis* R but devoid of activity for *L. casei* and for chicks.

It has also been shown that, for antianæmic activity, the presence of a side chain is unnecessary. Thus nutritional anæmia of rats<sup>128</sup> and of fish<sup>179</sup> can be cured by administration of xanthopterin, and ichthyopterin (fluoresceyanine) is curative in riboflavin-deficient rats and in aneurin-deficient rats and pigeons.<sup>129</sup> A number of other synthetic pterins also possess this interesting dual biological activity for the rat and the pigeon; certain micro-organisms (*Glaucoma*, *Polytomella Cæca*), however, are more exacting in their requirements, and are unable to utilise pterins in place of aneurin.<sup>129</sup>

In conclusion, two points of nomenclature should be mentioned. In the first place, recent comments<sup>177</sup> on the synthesis of pteroylglutamic

<sup>176</sup> J. J. Piffner, D. G. Calkins, E. S. Bloom, and B. L. O'Dell, *J. Amer. Chem. Soc.*, 1946, **68**, 1392.

<sup>177</sup> K. A. Jensen, *Dansk Tidsskr. Farm.*, 1946, **20**, 219; *Lancet*, 1946, *i*, 969; 1946, *ii*, 532, 680.

acid refer to this substance as folic acid, whereas the American workers consistently <sup>171, 174, 178</sup> use the names pteroylglutamic acid or liver *L. casei* factor when referring to their synthetic product. This distinction should clearly be retained for the present, because no announcement has been made of formal proof that the folic acid of H. K. Mitchell *et al.*<sup>165</sup> is identical with pteroylglutamic acid; indeed, B. L. Hutchings *et al.* have pointed out <sup>170</sup> that absorption spectra measurements indicate that folic acid is not identical with vitamin B<sub>12</sub>, vitamin B<sub>12</sub> conjugate, or the fermentation *L. casei* factor, and no modification of this view has appeared in literature available to the Reporter. Secondly, the American workers have departed from the established numbering of the pteridine nucleus (based on analogy with the purine ring-system), and have used a method <sup>162, 174</sup> based on lumazine <sup>175</sup> as the parent nucleus; this introduces an unnecessary complication, and the established nomenclature has been used throughout this Report.

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J. K. N. JONES.

M. S. LESSLIE.

C. W. SHOPPEE.

J. C. E. SIMPSON.

E. E. TURNER.

<sup>178</sup> R. B. Angier, *Dansk Tidsskr. Farm.*, 1946, **20**, 288.

<sup>179</sup> R. W. Simmons and E. R. Norris, *J. Biol. Chem.*, 1941, **140**, 679.

## BIOCHEMISTRY.

### I. INTRODUCTION.

THE continual development of biochemistry, reflected in its widening scope and greatly increased specialisation, has for some years made inevitable a gradual change in the nature of this section of the Reports. Whereas it was earlier possible to survey in outline almost all the year's principal advances, fewer subjects can now be included each year in the space available, unless their treatment is to be so superficial as to consist of little more than a catalogue.

In the present Report, therefore, a selection of the important developments is presented in the form of brief reviews, in some of which it has been found possible to include more reference to the general background than was possible in the older form of annual annotation. Difficulties due to the still delayed publication of war-time researches remain a considerable handicap to the Reporters, and for this reason a contemplated survey of the sulphhydryl enzymes could not be completed for inclusion in the present Report.

F. D.

### 2. BIOLOGICAL METHYLATION.\*

About one hundred years ago several cases of poisoning occurred in Germany and were ascribed to the use of arsenical pigments on wall-papers. Summaries of the earlier literature on this subject have been published by R. Abel and P. Buttenberg,<sup>1</sup> H. Huss,<sup>2</sup> and A. Maassen.<sup>3</sup> L. Gmelin<sup>4</sup> noticed a garlic odour in rooms where the symptoms had developed. This he ascribed to a volatile arsenic compound liberated from the damp and mouldy wall-paper. F. Selmi<sup>5</sup> suggested that the moulds produced hydrogen which, acting on the pigment, gave rise to arsine. A suggestion that the gas was arsine had already been made by Martin<sup>6</sup> in 1847 but without reference to mould action. In 1846 Basedow<sup>7</sup> suggested, but without experimental support, that the air of the rooms might contain cacodyl oxide,  $\text{Me}_2\text{As}\cdot\text{O}\cdot\text{AsMe}_2$ .

In 1891 B. Gosio<sup>8</sup> exposed a potato-mash containing arsenious oxide to the air. It quickly became infected with moulds and bacteria and evolved a garlic odour. Some of the moulds were intensely active, especially one which Gosio named *Penicillium brevicaulis*—the modern name

<sup>1</sup> *Z. Hyg.*, 1899, **32**, 499.

<sup>2</sup> *Ibid.*, 1914, **76**, 361.

<sup>3</sup> *Arch. Kais. Gesund.*, 1902, **18**, 479.

<sup>4</sup> *Karlsruher Zeitung*, November 1839.

<sup>5</sup> *Ber.*, 1874, **7**, 1642.

<sup>6</sup> *Gazette Médicale*, 1847, Feb. 13, 130.

<sup>7</sup> *Schmidt's Jahrbuch*, 1846, **52**, 89.

<sup>8</sup> *Arch. Ital. Biol.*, 1893, **18**, 253, 298; *ibid.*, 1901, **35**, 201; *Ber.*, 1897, **30**, 1024.

\* Parts of this report are based on earlier articles by the author, particularly that published in *Chem. Reviews*, 1945, **36**, 315.

is *Scopulariopsis brevicaulis*. Other organisms which exhibited this phenomenon were *Aspergillus glaucus*, *A. virens*, and *Mucor Mucedo*. C. Thom and K. B. Raper<sup>9</sup> extended this list to include *A. fischeri*, *A. sydowi*, and a few soil organisms.

B. Gosio<sup>8</sup> elaborated a biological method for the detection of traces of arsenic in aqueous extracts of various materials. The evaporated extract was added to a slice of sterile potato previously inoculated with *S. brevicaulis*. After a few hours at 25—30° inorganic arsenic could be detected by the production of a garlic odour. H. R. Smith and E. J. Cameron<sup>10</sup> state that one-millionth of a gram of arsenious oxide in one gram of material can thus be recognised.

By passing "Gosio-gas" from arsenical cultures of *S. brevicaulis* through a hot tube, Gosio concluded that the gas contained an alkylarsine. P. Biginelli<sup>11</sup> aspirated the gas through mercuric chloride in dilute hydrochloric acid. The resulting precipitate was assigned the composition  $\text{AsHEt}_2 \cdot 2\text{HgCl}_2$ , and Biginelli concluded that the gas was diethylarsine. P. Klason,<sup>12</sup> from Biginelli's analyses and some further work, regarded it as diethylarsine oxide. N. Wigren<sup>13</sup> synthesised both these compounds and showed that their behaviour towards acid mercuric chloride (Biginelli's solution) was different from that of Gosio gas.

Owing to the uncertainty regarding the nature of Gosio-gas work was commenced by Challenger *et al.* in 1931. Four strains of *S. brevicaulis* were employed.

Sterile aqueous solutions of various arsenic compounds were added to bread cultures of *S. brevicaulis* arranged in series. Sterile air was passed through and volatile arsenic compounds absorbed in Biginelli's solution. Using arsenious oxide (0.2—0.25% in the bread) two different deposits were obtained according to the concentration of the mercuric chloride, consisting of the di- and the mono-mercurichloride of trimethylarsine,  $\text{AsMe}_3 \cdot 2\text{HgCl}_2$  and  $\text{AsMe}_3 \cdot \text{HgCl}_2$ . Gosio-gas is therefore trimethylarsine.<sup>14</sup> Direct comparison with an authentic specimen confirmed this conclusion. With sodium methylarsonate,  $\text{AsMeO}(\text{ONa})_2$  (1—1.5% in the bread), or sodium cacodylate,  $\text{AsMe}_2\text{O} \cdot \text{ONa}$  (0.1—0.3% in bread) (free from inorganic arsenic), the evolved gas gave the same mercurichloride.

The identity of Gosio-gas was then confirmed by several observations. By absorption in nitric acid trimethylhydroxyarsonium nitrate,  $\text{AsMe}_3(\text{OH}) \cdot \text{NO}_3$ , and the corresponding picrate were prepared, identical with those obtained from the synthetic arsine. Gosio-gas with alcoholic benzyl chloride gave a quaternary salt and thence benzyltrimethylarsonium picrate.

Evans *et al.*<sup>15</sup> suggest a bimolecular structure for trimethylarsine dimercurichloride.

<sup>9</sup> *Science*, 1932, **76**, 548.

<sup>10</sup> *Ind. Eng. Chem. (Anal.)*, 1933, **5**, 400.

<sup>11</sup> *Gazzetta*, 1901, **31**, 58.

<sup>12</sup> *Ber.*, 1914, **47**, 2634.

<sup>13</sup> *Annalen*, 1924, **437**, 285.

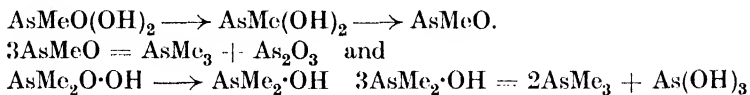
<sup>14</sup> F. Challenger, (Miss) C. Higginbottom, and L. Ellis, *J.*, 1933, 95.

<sup>15</sup> R. C. Evans, F. G. Mann, H. S. Peiser, and D. Purdie, *J.*, 1940, 1215.



*Alkylarsonic Acids and S. brevicaulis.*

It seemed possible that the mould might cause fission of the arsenic-carbon link in sodium methylarsonate and cacodylate giving inorganic arsenic, or that the trimethylarsine might have arisen by reduction followed by dismutation, thus :



With sodium ethylarsonate in bread cultures of the mould dimethylethylarsine,  $\text{AsMe}_2\text{Et}$ , was evolved and identified as the mercurichloride, thus eliminating both these possibilities.

Absorption in benzyl chloride yielded benzyldimethylethylarsonium chloride and in nitric acid dimethylethylhydroxyarsonium nitrate which were characterised as the picrates. This reaction was then studied further.<sup>16</sup> Addition of (a) diethylarsonic acid,  $\text{AsEt}_2\text{O}\cdot\text{OH}$ , (b) *n*-propylarsonic acid, and (c) allylarsonic acid,  $\text{CH}_2\cdot\text{CH}\cdot\text{CH}_2\cdot\text{AsO(OH)}_2$ , to similar cultures of the same strain of the mould in concentrations varying from 0.2 to 0.5% gave mixed methylated arsines.

From (a) methyl-diethylarsine was obtained and from (b) dimethyl-*n*-propylarsine. This arsine was also obtained with methyl-*n*-propylarsonic acid and *S. brevicaulis*. It was identified as the dimercurichloride and as dimethyl-*n*-propylhydroxyarsonium picrate. Ethyl-*n*-propylarsonic acid gave methylethyl-*n*-propylarsine, and (c) gave dimethylallylarsine,  $\text{CH}_2\cdot\text{CH}\cdot\text{CH}_2\cdot\text{AsMe}_2$ , characterised as the dimercurichloride and as benzyldimethylallylarsonium picrate.

*Methylation of Inorganic Compounds of Selenium and Tellurium.*

O. Rosenheim<sup>17</sup> showed that, when *S. brevicaulis* was grown upon sterile bread containing inorganic compounds of selenium and tellurium, unpleasant odours were evolved. The substances responsible were not identified. A. Maassen,<sup>18</sup> judging entirely from odour, stated that the volatile products were diethyl selenide and diethyl telluride. He also examined the breath of animals injected with inorganic selenites and tellurites, and believed that here the odour was due to dimethyl selenide and dimethyl telluride (see also Japha<sup>19</sup>). A similar conclusion on equally unsatisfactory evidence had been reached as regards animals injected with tellurium compounds by F. Hofmeister.<sup>20</sup> Maassen concluded therefore that the animal body deals with compounds of selenium and tellurium differently from the organism of the mould.

*Methylation of Inorganic Compounds of Selenium.*—The gas evolved from Rosenheim's cultures containing selenium compounds was identified by

<sup>16</sup> F. Challenger and L. Ellis, *J.*, 1935, 396; F. Challenger and A. A. Rawlings, *J.*, 1936, 264.

<sup>17</sup> *Proc.*, 1902, 138.

<sup>19</sup> Dissertation, Halle, 1842.

<sup>18</sup> *Arb. Kais. Gesund.*, 1902, 18, 479.

<sup>20</sup> *Arch. exp. Path. Pharm.*, 1894, 33, 198.

F. Challenger and H. E. North.<sup>21</sup> The volatile products from several cultures of two different strains of *S. brevicaulis* on bread containing sodium selenate or selenite were aspirated through absorbents and characterised as dimethyl selenide mercurichloride and mercuribromide,  $\text{SeMe}_2, \text{HgX}_2$ , dimethylhydroxyselenonium nitrate, dimethyl selenide  $\alpha$ -platinochloride, and benzyl dimethylselenonium chloride, isolated as the picrate.

*Methylation of Inorganic Compounds of Tellurium.*—The odour exhaled by animals receiving inorganic derivatives of tellurium was first observed by C. Gmelin.<sup>22</sup> A. Hansen,<sup>23</sup> on administration of potassium tellurite to dogs or men, detected a garlic odour in the breath after a few minutes. This lasted for weeks, and the persons in question were obliged to forsake the society of their fellows. See also W. Blyth,<sup>24</sup> who mentions the phenomenon of "bismuth breath", formerly well known to pharmacists and due to the presence of traces of tellurium in medicinal preparations of bismuth. Further details are given by G. Brownen,<sup>25</sup> E. A. Letts,<sup>26</sup> and A. Reissert.<sup>27</sup> In no case was the odorous substance satisfactorily identified.

(Miss) M. L. Bird and F. Challenger<sup>28</sup> aspirated the product evolved from test-tube cultures of *S. brevicaulis* on bread containing potassium tellurite through about 5 c.c. of reagent. Oxidation was thus diminished and dimethyl telluride mercurichloride was obtained and converted into dimethyl telluride dibromide. Absorption in alcoholic iodine gave dimethyl telluride di-iodide.

The mould gas is therefore dimethyl telluride, and Maassen's statement that it consists of the diethyl compound is incorrect. This conclusion was also confirmed with liquid cultures on 2% glucose-Czapek-Dox medium.

#### *Methylating Capacities of certain Penicillia.*

A green mould which appeared as a spontaneous infection on bread crumbs moistened with a tellurite solution was found by Dr. Thom of the U.S. Department of Agriculture, Washington, to be closely allied to *Penicillium notatum*, Westling. Cultures on bread and on 2% glucose-Czapek-Dox medium containing tellurite evolved dimethyl telluride which was identified as before and as benzyl dimethyltelluronium picrate.

*P. chrysogenum* Thom in tellurite-bread cultures gave dimethyl telluride, but only a faint odour was observed with *P. notatum*. Both organisms readily gave dimethyl selenide in bread cultures containing selenite or selenate. This was also produced in bread-selenate cultures by the "green mould".

In bread cultures none of the three green *Penicillia* gives trimethylarsine with arsenious acid, but all convert sodium methylarsonate into trimethyl-

<sup>21</sup> *J.*, 1934, 68.

<sup>22</sup> "Wirkungen . . . auf den tierischen Organismus", Tübingen, 1824, 43.

<sup>23</sup> *Annalen*, 1853, 86, 213.

<sup>24</sup> "Poisons : their Effects and Detection", 1884, 588.

<sup>25</sup> *Pharm. J.*, 1876, 6, 561.

<sup>26</sup> *Ibid.*, 1878, 9, 405, 407.

<sup>27</sup> *Amer. J. Pharm.*, 1884, 56, 177.

<sup>28</sup> *J.*, 1939, 163.

arsine which is also produced in similar cultures of *P. chrysogenum* and *P. notatum* containing sodium cacodylate. Although methyl groups are present in the substrate, dismutation appears to be excluded because bread cultures of *P. chrysogenum* convert sodium allylarsionate into dimethylallylarsine,  $\text{CH}_2\cdot\text{CH}\cdot\text{CH}_2\cdot\text{AsMe}_2$ .

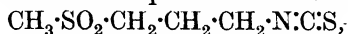
*Fission of the Disulphide Link in Dialkyl Disulphides by S. brevicaulis and Methylation of the Alkyl S-Group.*

Attempts were made to obtain dimethyl sulphide by the use of two different strains of *S. brevicaulis*. Negative results<sup>21</sup> were obtained with sulphur, sodium sulphite, sodium thiosulphate, sodium tetrathionate, thiourea, thiodiglycollic acid and its sodium salt, and sodium formaldehyde-sulphoxylate ("rongalite"), and also with sodium ethanesulphonate and ethanesulphinic acid, the last-named compound in liquid cultures.

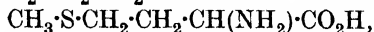
This was somewhat surprising in view of the experiments of J. Pohl,<sup>29</sup> who noticed a leek-like odour in the breath of animals receiving injections of thiourea. The odorous product was non-reactive to sodium hydroxide or mercuric cyanide, and was therefore not an alkanethiol. It was, however, absorbed by sulphuric acid and gave a precipitate with mercuric chloride. Pohl therefore concluded that the product was an alkyl sulphide. A similar odour is exhaled by patients suffering from hyperthyroidism and receiving thiourea.<sup>30</sup>

C. Neuberg and P. Grosser<sup>31</sup> stated that the precursor of the diethyl sulphide which was shown by J. J. Abel<sup>32</sup> to be evolved on warming the urine of dogs with alkali is methyldiethylsulphonium hydroxide; also that administration of diethyl sulphide to dogs gives rise to this compound. Experimental details are lacking.

The occurrence in nature of compounds such as cheirolin,



erysolin,  $\text{CH}_3\cdot\text{SO}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{N}\cdot\text{C}\cdot\text{S}$ <sup>33</sup> and methionine,



demonstrates the possibility of a biological methylation of sulphur. The relation of methionine to cysteine and to cystine suggested that compounds containing the -SH or -S-S- links might be more amenable to the methylating action of the mould.

Disulphides ( $\text{R}\cdot\text{S}\cdot\text{S}\cdot\text{R}$ ;  $\text{R} = \text{Et}$  or  $n\text{-Pr}$ ) with excess of saturated aqueous mercuric chloride give insoluble compounds  $\text{SR}\cdot\text{HgCl}\cdot\text{HgCl}_2$ ,<sup>34</sup> identical with those obtained from the alkanethiols. With dimethyl and diethyl disulphides the soluble products were shown to be the alkanesulphinic

<sup>29</sup> *Arch. exp. Path. Pharm.*, 1904, **51**, 341.

<sup>30</sup> References given by F. Challenger, *Chem. Reviews*, 1945, **36**, 333.

<sup>31</sup> *Centr. Bl. Physiol.*, 1905—1906, **19**, 316.

<sup>32</sup> *Z. physiol. Chem.*, 1894, **20**, 253.

<sup>33</sup> For references see E. F. Armstrong and K. F. Armstrong, "The Glycosides", 1931, 66.

<sup>34</sup> F. Challenger and A. A. Rawlings, *J.*, 1937, 868.

acids,  $\text{RSO}_2\text{H}$ , formed by dismutation of the sulphenic acid,  $\text{SR}\cdot\text{OH}$ . The sulphinic acids were characterised by Blackburn and Challenger<sup>35</sup> as the *p*-nitrobenzyl alkyl sulphones.

#### *S. brevicaulis and Dialkyl Disulphides.*

The behaviour of disulphides to mercuric chloride having been established, dialkyl disulphides (methyl to *n*-amyl) were added in dilute aqueous suspension to bread cultures. The volatile products contained the alkanethiol,  $\text{SHR}$  [absorbed in mercuric cyanide giving  $(\text{SR})_2\text{Hg}$ ], the unchanged disulphide,  $\text{R-S-S-R}$ , and the methyl alkyl sulphide,  $\text{SRMe}$ . The precipitates obtained with mercuric chloride were mixtures of the mercuric chloride addition product of the methyl alkyl sulphide with varying amounts of  $\text{RSHgCl}$ ,  $\text{HgCl}_2$ , arising from fission of  $\text{RS}\cdot\text{SR}$ . On treatment of these mixtures with sodium hydroxide, pure methyl alkyl sulphide was evolved; this was converted into the mercurichloride, the benzylmethylalkylsulphonium picrate, or the double compound with platinous chloride.

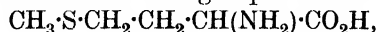
The fission of the disulphide link by *S. brevicaulis* appears, therefore, to be a general reaction of the simple aliphatic disulphides.<sup>34, 35</sup>

#### *Methylation of Inorganic Sulphate by Schizophyllum commune.*

Birkinshaw, Findlay, and Webb<sup>36</sup> have shown that the wood-destroying fungus *Schizophyllum commune*, Fr., when grown on an aqueous medium containing glucose, inorganic salts, and a trace of "marmite", converts inorganic sulphate into methanethiol. This was characterised as mercury thiomethoxide  $(\text{SMe})_2\text{Hg}$ . Traces of hydrogen sulphide are also produced. This is the only recorded instance of the mycological methylation of inorganic sulphur. Although *S. brevicaulis* forms dimethyl selenide from inorganic selenium compounds no methylselenothiol is produced. F. Challenger and P. T. Charlton<sup>37</sup> find that dimethyl sulphide and disulphide accompany the methanethiol evolved by *S. commune*. The disulphide probably arises by aerial oxidation of the thiol.

#### *Mycological Fission of the Carbon-Sulphur Link.*

The methanethiol evolved by cultures of *S. commune* might possibly be formed by fission of the terminal  $\text{SMe}$  group of methionine,



synthesised by the fungus. Addition of *dl*-methionine to cultures of *S. commune*, however, gave only traces of methanethiol. The question arose whether a similar stability would be exhibited by methionine in bread cultures of *S. brevicaulis*. Actually the amino-acid was readily converted into methanethiol and dimethyl sulphide. Under identical conditions *S*-methyl-, -ethyl-, and -*n*-propylcysteine gave the corresponding alkanethiol and methyl alkyl sulphide.<sup>37</sup> This fission of the C-S link appears to be a

<sup>35</sup> S. Blackburn and F. Challenger, *J.*, 1938, 1872.

<sup>36</sup> J. H. Birkinshaw, W. P. K. Findlay, and R. A. Webb, *Biochem. J.*, 1942, **36**, 526.

<sup>37</sup> *J.*, 1947, 424.

new type of mycological action. The mechanism may be reductive giving homoalanine as the other primary product, or hydrolytic when homoserine,  $\text{CH}_2(\text{OH})\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{CO}_2\text{H}$ , would be formed.

The alkanethiols obtained in *S. brevicaulis* cultures from methionine and the *S*-alkylcysteines may be formed by the fission of the corresponding keto-acids rather than directly from the amino-acids. Methionine is converted by kidney or liver slices<sup>38</sup> and also on feeding to rats<sup>39</sup> into the keto-acid,  $\text{CH}_3\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CO}\cdot\text{CO}_2\text{H}$ . This keto-acid readily yields methanethiol with dilute acids or alkalis.

The fission of the C-SMe link in methionine and the *S*-alkylcysteines by mould cultures has only one other biological counterpart, namely the—probably reversible—fission of the unsymmetrical amino-acid cystathionine,  $\text{CO}_2\text{H}\cdot\text{CH}(\text{NH}_2)\cdot\text{CH}_2\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{CO}_2\text{H}$ .<sup>40</sup> In presence of rat liver or kidney slices or saline extracts of rat liver this gives cysteine and possibly homoserine or its phosphoric ester.<sup>41</sup> Cystathionine appears to play an important part in the biological conversion of methionine into cysteine.<sup>42, 43</sup>

#### *Oxidative Demethylation of N-methyl Compounds.*

K. Hess *et al.*<sup>44</sup> showed that *N*-methylated keto-acids derived from pyrrolidine and piperidine on treatment with phenylhydrazine or semicarbazide yield secondary alcohols, the  $>\text{NMe}$  group giving rise to  $>\text{NH}$  and the phenylhydrazone or semicarbazone of formaldehyde.

Recent investigations using isotopic indicators show that certain methylated amino-acids or amines undergo demethylation by animals or animal tissues. Some earlier work may first be cited.

Dimethylaniline yields the glycuronate of *p*-methylaminophenol in rabbits.<sup>45</sup> Some methylaniline was detected in the urine. Demethylation of dimethylaniline to aminophenol is also effected by dogs. M. Lewis and R. A. Tager<sup>46</sup> state that *N*-methyl- and *NN*-dimethyl-sulphanilamides are demethylated when administered to men or mice.

E. S. Stevenson, K. Dobriner, and C. P. Rhoads<sup>47</sup> found that in rats demethylation of *p*-dimethylaminoazobenzene occurs, accompanied by fission and reduction of the azo-linkage, and that the urine contains *p*-aminophenol, *N*-acetyl-*p*-aminophenol, *p*-phenylenediamine, and *NN'*-diacetyl-*p*-phenylenediamine.

<sup>38</sup> E. Borek and H. Waelsch, *J. Biol. Chem.*, 1941, **141**, 99.

<sup>39</sup> H. Waelsch, *ibid.*, **140**, 313.

<sup>40</sup> G. B. Brown and V. du Vigneaud, *ibid.*, **137**, 611; V. du Vigneaud, G. B. Brown, and J. P. Chandler, *ibid.*, 1942, **143**, 59.

<sup>41</sup> F. Binkley and V. du Vigneaud, *ibid.*, **144**, 507; F. Binkley, W. P. Anslow, and V. du Vigneaud, *ibid.*, **143**, 559.

<sup>42</sup> D. Stetten, *ibid.*, **144**, 501.

<sup>43</sup> V. du Vigneaud, G. W. Kilmer, J. R. Rachele, and (Miss) M. Cohn, *ibid.*, 1944, **155**, 645.

<sup>44</sup> *Ber.*, 1913, **46**, 4104; 1915, **48**, 1886; 1917, **50**, 344, 351, 385.

<sup>45</sup> F. Horn, *Z. Physiol. Chem.*, 1936, **242**, 23; 1936, **238**, 84.

<sup>46</sup> *Yale J. Biol. Med.*, 1940, **13**, 111.

<sup>47</sup> *Cancer Research*, 1942, **2**, 160.

K. Bloch and R. Schoenheimer<sup>48</sup> fed rats with (a) isotopic glycine and (b) isotopic sarcosine (*N*-methylglycine). Glycine was isolated from the tissue protein as the trioxalatochromate, the concentration of isotopic nitrogen being almost identical in each case. It is suggested that sarcosine is demethylated in the tissues without loss of nitrogen, and sarcosine can replace glycine as a detoxicating agent when benzoic acid is fed to rabbits. *N*-ethylglycine causes no increase in the rate of excretion of hippuric acid when administered with benzoate to rabbits, suggesting that de-ethylation is at any rate a much slower process.<sup>49</sup> The oxidative demethylation of sarcosine to formaldehyde and glycine has been established with broken cell preparations of the liver of cats and rabbits.<sup>50</sup> Other *N*-methylamino-acids are not necessarily metabolised in the same way, *N*-methylalanine giving pyruvic acid and methylamine with amino-acid oxydase.<sup>51</sup>

du Vigneaud *et al.*<sup>52</sup> have shown that, unlike certain closely related compounds (which do not eliminate a methyl group as formaldehyde), sarcosine exerts no methylating action in animal experiments (see p. 274).

*N*<sup>1</sup>-methylnicotinamide (see p. 273) is stated<sup>53</sup> to undergo demethylation to nicotinic acid in rats when administered with glycocyamine. No increase in the urinary output of creatine and creatinine was observed.

In a comprehensive review on biological methylation, S. J. Bach<sup>54</sup> has discussed the evidence available before 1945 for the demethylation of purines in animals or animal tissues and concludes that the question is still controversial. Caffeine does not take part in transmethylation<sup>55</sup> (see p. 274).

From a recent study of the metabolism of the mono-, di-, and tri-methyluric acids in the Dalmatian dog and albino rat, V. G. Myers and R. F. Hanzal<sup>56</sup> conclude that 3-methyluric acid appears to be completely demethylated and converted into uric acid; the 1 : 3 : 7 derivative is partially demethylated in position 7, and the 1 : 3 compound is largely unchanged though some demethylation may occur at 3.

#### *Mechanism of Biological Methylation.*

Three mechanisms have been suggested to account for the phenomena of biological methylation and the evidence has been fully discussed.<sup>57</sup> The

<sup>48</sup> *J. Biol. Chem.*, 1940, **135**, 99.

<sup>49</sup> L. P. Abbot and H. B. Lewis, *J. Biol. Chem.*, 1939, **131**, 479; 1941, **137**, 535.

<sup>50</sup> P. Handler, M. L. C. Bernheim, and J. R. Klein, *J. Biol. Chem.*, 1941, **138**, 211; compare K. Hess, reference 44.

<sup>51</sup> D. Keilin and E. F. Hartree, *Proc. Roy. Soc.*, 1936, *B*, **119**, 114.

<sup>52</sup> V. du Vigneaud, J. P. Chandler, A. W. Moyer, and D. M. Keppel, *J. Biol. Chem.*, 1939, **131**, 57.

<sup>53</sup> V. A. Najjar and (Miss) C. C. Deal, *ibid.*, 1946, **162**, 741.

<sup>54</sup> *Biol. Rev.*, 1945, **20**, 158, 167.

<sup>55</sup> A. W. Moyer and V. du Vigneaud, *J. Biol. Chem.*, 1942, **143**, 373.

<sup>56</sup> *Ibid.*, 1946, **162**, 309.

<sup>57</sup> F. Challenger, *Chem. and Ind.*, 1942, **61**, 399, 413, 456; *Chem. Reviews*, 1945, **36**, 315.

first of these involves the interaction of acetic acid with the compound undergoing methylation and is based on the well-known "cacodyl reaction". It need not be further considered here. The second, the formaldehyde hypothesis, merits further discussion on purely chemical grounds, and also in view of the production of formaldehyde by oxidative demethylation under biological conditions. No direct evidence for this hypothesis is available on the biological side, however.

For the third hypothesis—that of transmethylation—conclusive evidence has been obtained from animals, though not yet from moulds. The mechanism by which the methyl group is transferred still remains obscure.

*The Formaldehyde Hypothesis.*—In moulds and animals any formaldehyde involved in methylation reactions is presumably of secondary origin and even in plants some may arise by the demethylation of NMe groups, or by oxidation of purines to uric acid which, by way of allantoin, can give rise enzymically to glyoxylic acid,  $\text{CHO}\cdot\text{CO}_2\text{H}$ , and urea as shown by M. R. Fosse and A. Brunel and their colleagues.<sup>58</sup>

It was not possible to apply a crucial test to the formaldehyde hypothesis as regards moulds. In its application to the production of trimethylarsine from arsenious acid this postulates the formation of hydroxymethylarsonic acid,  $\text{CH}_2(\text{OH})\cdot\text{AsO}(\text{OH})_2$ , as the first stage, followed by reduction to methylarsonic acid,  $\text{Me}\cdot\text{AsO}(\text{OH})_2$ . After further reduction to  $\text{Me}\cdot\text{As}(\text{OH})_2$  the isomeric form  $\text{Me}\cdot\text{AsO}(\text{OH})\text{H}$  might be expected to react again with formaldehyde, repetition of the process yielding cacodylic acid,  $\text{Me}_2\text{AsO}\cdot\text{OH}$ , and finally trimethylarsine. Hydroxymethylarsonic acid could not be synthesised, and its homologue  $\text{CH}_2(\text{OH})\cdot\text{CH}_2\cdot\text{AsO}(\text{OH})_2$  in bread cultures of the mould gave no volatile product. Had reduction of the  $\beta$ -hydroxyl group occurred the formation of dimethylethylarsine would have been expected.<sup>58a</sup>

If selenious and tellurous acids can react as  $\text{SeO}_2(\text{OH})\text{H}$  and  $\text{TeO}_2(\text{OH})\text{H}$  the formaldehyde hypothesis can explain their conversion into dimethyl selenide and dimethyl telluride in mould cultures. The work of W. Strecker and W. Daniel<sup>59</sup> raises doubt as to whether selenious acid can react in this form. See, however, J. Loevenich, H. Fremdling, and M. Föhr<sup>60</sup> who find that  $\beta$ -naphthylseleninic acid,  $\text{C}_{10}\text{H}_7\cdot\text{SeO}_2\text{H}$ , gives a normal ester and also a selenone.

As applied to the fission of disulphides and methylation of the resulting thiol, the formaldehyde hypothesis demands the formation of  $\text{RS}\cdot\text{CH}_2\cdot\text{OH}$ . Compounds of this type have been described<sup>61</sup> but are unstable and easily

<sup>58</sup> Numerous references cited in *Chem. Reviews*, 1945, **36**, 338.

<sup>58a</sup> F. Challenger, C. Higginbottom, and L. Ellis, *J.*, 1933, 95; F. Challenger and C. Higginbottom, *Biochem. J.*, 1935, **29**, 1757.

<sup>59</sup> *Annalen*, 1928, **462**, 186.

<sup>60</sup> *Ber.*, 1929, **62**, 2856.

<sup>61</sup> T. G. Levi, *Gazzetta*, 1932, **62**, 775; F. Challenger and A. A. Rawlings, *J.*, 1937, 868.

hydrolysed. The compound  $\text{CH}_3\cdot\text{CH}_2\cdot\text{S}\cdot\text{CH}_2\cdot\text{OH}$  could not be freed from traces of ethanethiol and so its capability of reduction to  $\text{SMeEt}$  in mould cultures could not be determined.<sup>61</sup>

*The Transfer of a Methyl Group.*—The transfer of a methyl group from some methylated compound such as choline or betaine was suggested by O. Riesser<sup>62</sup> to explain the production of creatine and of alkylated (presumably methylated) derivatives of selenium and tellurium in animals.<sup>63</sup>

F. Challenger and (Miss) C. Higginbottom<sup>64</sup> and F. Challenger, P. Taylor, and B. Taylor<sup>65</sup> found that sodium sulphite, organic disulphides, sodium selenite, and sodium tellurite when heated with betaine (free from hydrochloride, to avoid the formation of methyl chloride) and in absence of sodium formate, yielded dimethyl sulphide, methyl alkyl or methyl aryl sulphide, dimethyl selenide, and dimethyl telluride. All these products were characterised. The last three reactions exhibit a parallel with the behaviour of these substances in cultures of *S. brevicaulis* (see pp. 265, 267). R. Willstätter<sup>66</sup> found that, on heating, betaine forms methyl dimethyl-aminoacetate,  $\text{Me}_2\text{N}\cdot\text{CH}_2\cdot\text{CO}_2\text{Me}$ , a reaction clearly involving the migration of a methyl group.<sup>67</sup> It was suggested by F. Challenger<sup>68</sup> that these pyrogenic reactions might proceed as follows : (1)  $\text{Me}_3\text{N}^+\cdot\text{CH}_2\cdot\text{COO}^- + \text{Na}_2\text{SeO}_3 = \text{Me}_2\text{N}\cdot\text{CH}_2\cdot\text{CO}_2\text{Na} + \text{MeSeO}_3\text{Na}$ . With selenites and tellurites a quaternary salt is possibly first formed. The dimethyl selenide presumably arises by decomposition of the sodium methaneselenonate. (2)  $\text{Me}_3\text{N}^+\cdot\text{CH}_2\cdot\text{COO}^- + \text{RS}\cdot\text{SR} = \text{Me}_2\text{N}\cdot\text{CH}_2\cdot\text{CO}_2\text{SR} + \text{RSM}$ . Under similar conditions primary aromatic amines yielded *N*-monomethyl derivatives.

In the absence of any evidence as to the kinetics of these pyrogenic betaine decompositions it is impossible to say whether a free methyl ion is concerned in the reactions.

Experimental evidence is equally lacking as regards the kinetics of the production of methyl derivatives by living cells. Considering first a unimolecular mechanism of type  $\text{S}_\text{N}1$  it is noticed that almost all the compounds which undergo methylation by moulds or animals can give negative ions, which contain unshared electrons, so that co-ordination of a positive methyl group would give a neutral molecule.<sup>69</sup> This could then undergo reduction and ionisation followed by further co-ordination of a  $\text{CH}_3^+$  radical.

*Methylation of Arsenic, Selenium, and Tellurium Compounds.*—The

<sup>62</sup> *Z. physiol. Chem.*, 1913, **86**, 440.

<sup>63</sup> See F. Hofmeister, *Arch. exp. Path. Pharm.*, 1894, **33**, 198.

<sup>64</sup> *Biochem. J.*, 1935, **29**, 1757.

<sup>65</sup> *J.*, 1942, 48.

<sup>66</sup> *Ber.*, 1902, **35**, 584.

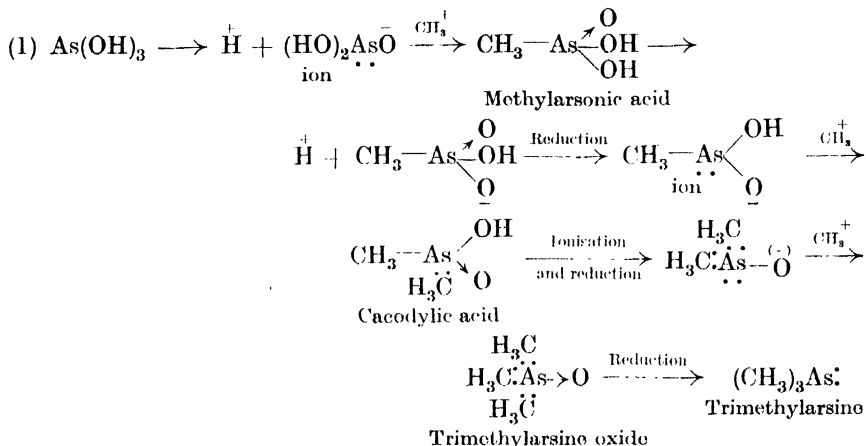
<sup>67</sup> Compare also H. T. Straw and H. T. Cranfield, *J. Soc. Chem. Ind.*, 1936, **55**, 40 T.

<sup>68</sup> *Chem. and Ind.*, 1942, **61**, 413, 456.

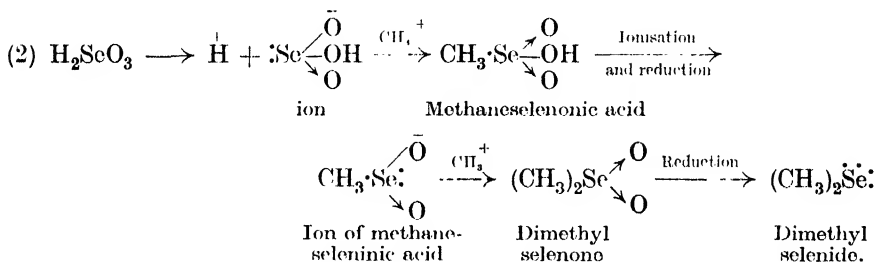
<sup>69</sup> F. Challenger, *Chem. Reviews*, 1945, **36**, 341, 347; E. D. Hughes and C. K. Ingold, *J.*, 1933, 1571; J. L. Gleave, E. D. Hughes, and C. K. Ingold, *J.*, 1935, 236.



mechanism suggested by the Leeds school <sup>69</sup> may be illustrated in the case of arsenious and selenious acids :



The suggested intermediate compounds have not been detected in mould cultures, but they all yield trimethylarsine when present in bread cultures of *S. brevicaulis*.



The postulated intermediate selenium compounds have not been detected in the media, but (Miss) M. L. Bird and F. Challenger <sup>70</sup> showed that *S. brevicaulis* and certain *Penicillia* convert methane-, ethane-, and propane-1-seleninic acids, RSeO<sub>2</sub>H, into dimethyl, methyl ethyl, and methyl *n*-propyl selenides, RSeMe, as required by the suggested mechanism, thus :



They point out, however, that direct reduction of the seleninic acid to selenothiol, R·SeH, might occur followed by methylation to R·SeMe, thus avoiding the selenone stage.

Potassium methane-, ethane-, and propane-1-selenonates, <sup>71</sup> RSeO<sub>2</sub>·OK, in cultures of the same moulds gave only dimethyl selenide, owing to break-down of the selenonate giving R·OH and KHSeO<sub>3</sub>. This observation does not necessarily invalidate the suggested mechanism since the methane-

<sup>70</sup> *J.*, 1942, 574.

<sup>71</sup> (Miss) M. L. Bird and F. Challenger, *J.*, 1942, 570.

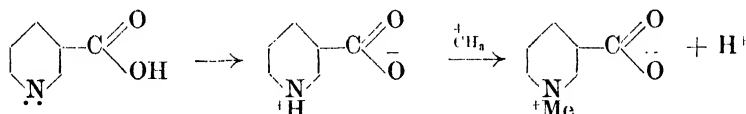
selenonic acid might be sufficiently stable, within the cell, to reach the next stage without hydrolysis.

*Methylation of Sulphur Compounds.*—The methyl alkyl sulphides obtained from dialkyl disulphides in cultures of *S. brevicaulis* may arise by ionisation of alkanethiol first produced, followed by co-ordination of  $\text{CH}_3$ , or this may occur before fission.<sup>72</sup>

Addition of sodium sulphite, methanesulphonate, or ethanesulphinate,  $\text{Et}\cdot\text{SO}_2\text{Na}$ , to liquid cultures of the mould gave no dimethyl or methyl ethyl sulphide. This might possibly be ascribed to the formation of methanesulphonic acid or of dimethyl or methyl ethyl sulphone by reactions analogous with those postulated for sodium selenite. Diethyl sulphone, unlike diethyl sulphoxide,<sup>73</sup> is not reduced to diethyl sulphide by *S. brevicaulis*, and sulphones, if formed, would probably accumulate, but the liquid culture media yielded no dimethyl or methyl ethyl sulphone. Methanesulphonic acid might also resist further reaction, when neither sulphone nor sulphide would be formed. Attempts to detect this acid in liquid cultures containing sodium sulphite failed.

*Methylation of Nitrogen Compounds.*—Co-ordination of a positive methyl ion would also explain the well-known conversion of neutral pyridine<sup>74</sup> and quinoline<sup>75</sup> into methylpyridinium and methylquinolinium hydroxides in the body of the dog.

The formation of trigonelline<sup>76</sup> or *N*<sup>1</sup>-methylnicotinamide<sup>77</sup> (see below) on administration of nicotinic acid to various animals can be explained in the same way.



One alternative to methylation by elimination of a positive methyl ion is a bimolecular reaction of the  $\text{S}_{\text{N}}2$  type.<sup>78</sup>

Since, however, this also ultimately involves the attachment of methyl to the unshared electrons of the metalloid the formulations on pp. 271—272 may be retained for convenience in representing the suggested intermediate stages in the methylation process. It is possible, however, that methyl may be transferred as a neutral radical. Attempts to obtain evidence of this by addition of sulphur, in powder or as a colloidal solution, or of finely

<sup>72</sup> F. Challenger, P. Taylor, and B. Taylor, *J.*, 1942, 48; F. Challenger, *Chem. Reviews*, 1945, **36**, 344.

<sup>73</sup> F. Challenger and H. E. North, *J.*, 1934, 68.

<sup>74</sup> W. His, *Arch. exp. Path. Pharm.*, 1887, **22**, 253.

<sup>75</sup> Y. Komori *et al.*, *J. Biochem. (Japan)*, 1926, **6**, 21, 163; S. Tamura, *Chem. Abstracts*, 1925, **19**, 2705.

<sup>76</sup> D. Ackerman, *Z. Biol.*, 1912, **59**, 17.

<sup>77</sup> J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.*, 1942, **142**, 401; 1943, **150**, 395.

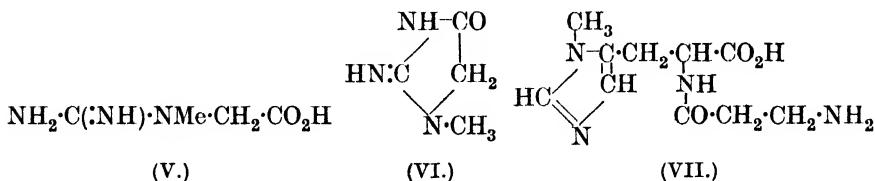
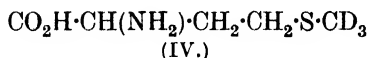
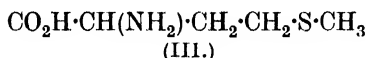
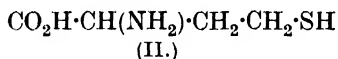
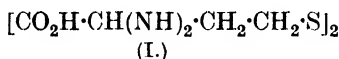
<sup>78</sup> J. L. Gleave, E. D. Hughes, and C. K. Ingold, *J.*, 1935, 236; E. D. Hughes and C. K. Ingold, *J.*, 1933, 1571.

divided mercury to cultures of *S. brevicaulis* gave negative results, no methylated compounds being detected.

As pointed out by Mr. J. H. Baxendale (private communication) the capture of a neutral methyl group by a negative ion, *e.g.*, arsenite, would give nine electrons on the arsenic atom, an unstable system which would act as a strong reducing agent, readily forming neutral methylarsonic acid,  $\text{MeAsO}(\text{OH})_2$ . This might possibly be concerned in the reducing actions which cultures of *S. brevicaulis* obviously exert upon the higher valencies of arsenic, selenium, and tellurium, inorganic arsenates, selenates, and tellurates yielding organic arsines, selenides, and tellurides.

*Transmethylation. Du Vigneaud's Experiments using Isotopic Indicators.*

*Transmethylation from Methionine and Choline.*—The suggestion that certain biological methylations in animals might be conditioned by methyl groups detached from choline or betaine<sup>62, 64, 65</sup> received support from the work of du Vigneaud and his colleagues. They have shown<sup>79</sup> that homocystine (I) can replace methionine (III) in the diet of the white rat only in presence of choline or betaine, which, however, produces the effect more slowly than choline. It was suggested that a methyl group is transferred from the nitrogen of choline or betaine to the sulphur of homocysteine (II) ("transmethylation") to give methionine and that the reaction might be reversible, methionine acting as a donor of methyl groups to a choline precursor.



Choline prevents a pathological condition known as fatty infiltration of the liver in rats. This is known as a lipotropic effect. It appeared possible that the growth observed in the dietary experiments might have been due simply to this particular effect of choline, the liver thus being enabled to remain healthy and to carry out methylation by some other means than a transference of methyl from choline.

This explanation was disproved when the choline was replaced by its ethyl analogue,  $\text{NEt}_3(\text{OH})\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$ , which also prevents fatty infiltration. This compound did not allow of the growth of rats on a choline—

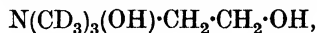
<sup>79</sup> J. P. Chandler and V. du Vigneaud, *J. Biol. Chem.*, 1940, **135**, 223; V. du Vigneaud, J. P. Chandler, and A. W. Moyer, *ibid.*, 1941, **139**, 917.

methionine-free diet containing homocystine. Du Vigneaud points out<sup>80</sup> that, had an ethyl group been transferred, ethionine [*S*-ethylhomocysteine,  $\text{SEt}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{CO}_2\text{H}$ ] would have been formed, and this was shown by H. M. Dyer<sup>81</sup> to be incapable of replacing methionine in the diet. Furthermore on feeding ethionine and choline to rats on a methionine-free diet no growth resulted, indicating that homocysteine is not formed from ethionine in the body. This stability of the S-Et link in ethionine recalls the difficulty experienced in the de-ethylation of ethylglycine in rabbits<sup>49</sup> or of certain *N*-ethylphenazine derivatives under purely chemical conditions.<sup>82</sup>

Du Vigneaud's transmethylation hypothesis was tested by the use of specimens of deuteromethionine (IV) containing (a) 83.6 and (b) 87.5 atom per cent. of deuterium in the methyl group. These were fed to rats kept on a methionine-choline-free diet.<sup>83</sup> Earlier work had shown that the deuterium content of the urinary creatinine (VI) closely follows that of the creatine (V) and choline of the tissues. The experiment with specimen (a) was, therefore, continued for 94 days until the methyl group of the creatinine contained 72.4 atom per cent. of deuterium. The animal was then killed and the choline isolated from the tissues as the chloroplatinate. The atom percentage of deuterium in the methyl groups of this choline was found to be 74.2, the corresponding figure for the tissue creatine being 73. These figures represent in all three cases approximately 85 per cent. of the theoretically possible amount of deuterium, assuming that all the methyl groups had come from the deuteromethionine. This figure is the "deuterium ratio", i.e., atom per cent. deuterium in methyl group of isolated compound/atom per cent. deuterium in methyl group of deuteromethionine administered  $\times 100$ . Oxidation of the choline to trimethylamine showed that all the deuterium was contained in the methyl groups.

It is concluded that these reactions are true transmethylations (the methyl group being transferred as a whole) and that they do not involve the oxidative elimination of dideuteroformaldehyde,  $\text{CD}_2\text{O}$ .<sup>44, 50</sup> On the formaldehyde theory of methylation dideuteroformaldehyde, if produced, would react with the amino-group of the choline precursor, presumably 2-hydroxyethylamine,<sup>84</sup> to give  $-\text{NH}\cdot\text{CD}_2\cdot\text{OH}$  which, on reduction in the organism, would give  $-\text{NH}\cdot\text{CD}_2\text{H}$  and not  $-\text{NH}\cdot\text{CD}_3$ . The deuterium content of each methyl group of the choline could not then rise above two-thirds of that in the methyl group of the methionine administered, i.e., the "deuterium ratio" would have a maximum at 66.6 per cent.

Du Vigneaud *et al.*<sup>85</sup> then administered trideuterocholine,



to rats, on a methionine-choline-free diet containing homocystine, for 23 and 56 days, respectively. On isolation of the creatine (V) from the tissues

<sup>80</sup> V. du Vigneaud, *Biol. Symposia*, 1941, **5**, 234.

<sup>81</sup> J. Biol. Chem., 1938, **124**, 519.

<sup>82</sup> H. McIlwain, *J.*, 1937, 1705.

<sup>83</sup> V. du Vigneaud, (Miss) M. Cohn, J. P. Chandler, J. R. Schenck, and (Miss) S. Simmonds, *J. Biol. Chem.*, 1941, **140**, 625.

<sup>84</sup> D. Stetten, *ibid.*, 1941, **140**, 143.

<sup>85</sup> *Ibid.*, 1943, **149**, 519.

the deuterium content was 24 and 29 per cent. of the theoretical maximum and the deuteromethyl group was detected in tissue methionine. The methyl groups of choline can therefore take part in transmethylation. This also occurs, to a lesser extent, when no homocystine is given or when ordinary methionine is given instead of homocystine.

The authors consider that homocysteine is formed from methionine by the animal, and that methionine is re-formed by means of the methyl group supplied by choline. Continuous synthesis of methionine therefore occurs although more than enough is supplied in the diet. When deuteromethionine and an adequate supply of ordinary choline were fed together, formation of choline from methionine was found to proceed nevertheless.

The occurrence of transmethylation has also been established in the rabbit<sup>86</sup> by the use of deuteromethionine (79 atom per cent. D in the methyl group), and analysis of the creatinine of the urine, the choline of the tissues and the anserine (VII) of the muscle. Later S. Simmonds and V. du Vigneaud<sup>87</sup> using the isotope technique, showed that the methyl group of dietary methionine can be used by man in the synthesis of choline and creatinine.

Du Vigneaud *et al.*<sup>88</sup> have investigated the relation of mono- and dimethylaminoethanol to choline and to transmethylation reactions. When the dimethyl compound was fed to young rats on a methyl-free basal diet containing homocystine, growth was not so good as when choline was fed—*i.e.*, methionine was less readily formed. However, deuterodimethylaminoethanol,  $(\text{CH}_2\text{D})_2\text{N}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$ , under similar conditions was readily converted into a deuteriocholine and thence into creatine by transmethylation. The ratio D in body choline/D in body creatine was large, whereas on feeding deuteromethionine to rats the ratio was almost unity.<sup>83</sup>

These results suggest that dimethylaminoethanol does not take part directly in transmethylation but that it can accept methyl groups supplied by methionine or some other methyl donor in the body, thus giving rise to choline and accounting for the limited growth-producing power. If so, it follows that choline, when engaging in transmethylation, releases only one methyl group giving dimethylaminoethanol. Experiments with deuteromethylaminoethanol,  $\text{CD}_3\cdot\text{NH}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$ , led to similar conclusions. The incapacity of the partly methylated aminoethanols to transfer their methyl groups is presumably due to the absence of the quaternary nitrogen atom which is present in choline and betaine.

Further work on the relation between choline and the methylaminoethanols has been carried out by Horowitz and his colleagues<sup>89</sup> using

<sup>86</sup> J. R. Schenck, (Miss) S. Simmonds, (Miss) M. Cohn, C. M. Stevens, and V. du Vigneaud, *J. Biol. Chem.*, 1943, **149**, 355. <sup>87</sup> *Ibid.*, 1942, **146**, 685.

<sup>88</sup> V. du Vigneaud, J. P. Chandler, (Miss) S. Simmonds, A. W. Moyer, and (Miss) M. Cohn, *ibid.*, 1946, **164**, 603.

<sup>89</sup> N. H. Horowitz and G. W. Beadle, *J. Biol. Chem.*, 1943, **150**, 325; N. H. Horowitz, D. Bonner, and (Miss) M. B. Houlahan, *ibid.*, 1945, **159**, 145; N. H. Horowitz, *ibid.*, 1946, **162**, 413.

*Neurospora crassa*. Two mutant strains of this organism have lost the ability to synthesise choline possessed by the wild type. One mutant strain produces methylaminoethanol but is unable to convert it into choline at the normal rate. It therefore accumulates and is to be regarded as a normal intermediate in choline synthesis. It was isolated as the picrolonate. The other mutant cannot synthesise methylaminoethanol but can methylate it to choline if an exogenous supply is available.

*Transmethylation from Betaine*.—Final proof that betaine takes part in transmethylation has now been obtained.<sup>90</sup> The experiments of du Vigneaud carried out with white rats on a methionine and choline-free diet containing homocystine<sup>79</sup> (see p. 274) pointed clearly in this direction. Stetten<sup>84</sup> showed that on administration of betaine containing <sup>15</sup>N to rats the concentration of this isotope in the glycine of the tissue-protein was almost as high as when isotopic glycine was fed, thus proving demethylation of the betaine. The fate of the methyl group was not rigidly established, but Stetten believed it to be captured by ethanolamine (arising from reduction of the glycine) thus yielding choline, which was found to contain the <sup>15</sup>N. Furthermore betaine is a lipotropic agent<sup>91</sup> (see p. 274) and also prevents the development of hæmorrhagic kidneys, activities which usually, though not invariably, indicate the presence of labile methyl.

V. du Vigneaud *et al.*<sup>90</sup> fed betaine labelled with deuteromethyl groups and <sup>15</sup>N to growing rats. Isotopic analyses of the choline and creatine isolated from the rat tissues showed betaine to be a very effective methyl donor. Methyl groups from dietary betaine appear in tissue choline almost as rapidly as they appear from dietary deuteriocholine. The disparity in the amounts of <sup>15</sup>N and of deuterium found in the tissues proves that the betaine molecule is not converted as a whole into choline.

Dimethylglycine containing deuterium in the methyl groups was fed to young rats. Transmethylation giving choline and creatine occurred only to a very slight extent. Dimethylglycine was also unable to prevent the incidence of hæmorrhagic kidneys.

The methyl group of dietary methionine appears more rapidly in creatine<sup>92</sup> than do those of dietary betaine. H. Borsook and J. W. Dubnoff found that methionine can serve as a methyl donor in the enzymatic synthesis *in vitro* of creatine from guanidoacetic acid (glycocylamine) by surviving liver tissue, but that choline can function in this system only in presence of homocystine.<sup>93</sup> The transfer of methyl groups from choline and betaine to form creatine possibly involves transmethylation first to methionine and then either directly or indirectly to creatine.

<sup>90</sup> V. du Vigneaud, (Miss) S. Simmonds, J. P. Chandler, and (Miss) M. Cohn, *J. Biol. Chem.*, 1946, **165**, 639.

<sup>91</sup> References in *Chem. Reviews*, 1945, **36**, 350.

<sup>92</sup> V. du Vigneaud, J. P. Chandler, (Miss) M. Cohn, and G. B. Brown, *J. Biol. Chem.*, 1940, **134**, 787.

<sup>93</sup> *Ibid.*, **132**, 559; **134**, 635; 1941, **138**, 389, 405; 1945, **160**, 635.

*α-Keto-acids from Derivatives of Cysteine and Methionine.*

J. L. Wood and V. du Vigneaud<sup>94</sup> find that the *S*-benzyl-*N*-methyl-derivatives of *l*-cysteine and *dl*-homocysteine lose their methyl groups when fed to rats and are excreted as the corresponding *S*-benzyl-*N*-acetyl-*l*-amino-acids. This is believed to occur through the *N*-free keto-acids, which are then re-aminated and acetylated,<sup>95</sup> because *d*-amino-acid oxidase and broken cell preparations of rat kidney and liver convert *dl*-*N*-methyl-methionine into the 1-keto-3-methylthiobutyric acid,<sup>96</sup>



P. Handler and (Miss) M. L. C. Bernheim<sup>97</sup> have shown that *d*(+)-methionine is about half as active as the *l*-isomer in promoting creatine synthesis by liver slices *in vitro*. Benzoic acid, which inhibits *d*-amino-acid oxidase, also prevents creatine synthesis (transmethylation) with *d*(+)-methionine, but not with the *l*-isomer. It is assumed, therefore, that *d*(+)-methionine must first be converted into the  $\alpha$ -keto-acid,  $\text{CH}_3\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CO}\cdot\text{CO}_2\text{H}$ . Whether this can undergo transmethylation as such, or only after reamination to *l*-methionine, has not been decided. It is, however, fully as active in creatine synthesis as methionine.

*Derivatives of Methionine.*

*dl*-Methionine sulphoxide and methylsulphonium iodide can replace methionine in the diet of the white rat, but *dl*-methionine sulphone cannot. This has a mycological parallel. Diethyl sulphoxide is readily reduced to diethyl sulphide in cultures of *S. brevicaulis*, whereas the sulphone is not.<sup>73</sup>

Neither the sulphoxide nor the sulphone appreciably increase the methylation of glycocyamine by liver slices.<sup>97</sup> The sulphoxide, however, exerts a lipotropic action in rats. Assuming that this is due to a transfer of methyl to a choline precursor (which has not been established), the inertness of the sulphoxide in Handler and Bernheim's experiments *in vitro* is surprising. These authors state "it appears probable that the intact animal possesses some mechanism whereby methionine sulphoxide may be reduced to the parent substance which may then be utilised for choline synthesis."

*Synthesis of Labile Methyl in the Body.*

From work summarised in this report the hypothesis arose that the animal organism is incapable of generating methyl groups for methylations and that methyl groups in a particular form such as methionine and choline must be present in the diet.

V. du Vigneaud, S. Simmonds, J. P. Chandler, and M. Cohn have recently presented evidence<sup>98</sup> for the synthesis of a small amount of labile methyl

<sup>94</sup> *J. Biol. Chem.*, 1946, **165**, 95.

<sup>95</sup> W. I. Patterson, H. M. Dyer, and V. du Vigneaud, *ibid.*, 1936, **116**, 277; M. W. Kies, H. M. Dyer, J. L. Wood, and V. du Vigneaud, *ibid.*, 1939, **128**, 207.

<sup>96</sup> P. H. Handler, F. Bernheim, and J. R. Klein, *ibid.*, 1941, **138**, 203

<sup>97</sup> *Ibid.*, 1943, **150**, 335.

<sup>98</sup> *Ibid.*, 1945, **159**, 755.

groups in the rat maintained on a diet adequate in labile methyl. V. du Vigneaud<sup>99</sup> occasionally found animals capable of showing some growth on a homocystine diet without added choline and the growth of rats on a similar methyl-free diet was reported by Bennett *et al.*<sup>100</sup> The authors raised the concentration of deuterium in the body water of two rats to about 3 atom per cent. by intraperitoneal injection of 99.5 per cent. D<sub>2</sub>O and maintained this by giving drinking water containing 4 atom per cent. of D<sub>2</sub>O for three weeks. The deuterium content of the choline chloroplatinate then isolated from the tissues indicated that 7.7 and 8.5 per cent. respectively of the choline-methyl was derived from the body water. It is very unlikely that a direct exchange reaction would cause the appearance of deuterium in the methyl groups under these conditions. The authors consider that the synthesis of methyl groups by intestinal bacteria is the most logical interpretation of their results.

#### *Methylsulphonium Compounds in Natural Products.*

It was suggested<sup>101</sup> in 1940 that sulphonium derivatives of methionine<sup>97</sup> might play a part in biological processes. The fission of the alkyl S-C link in methionine by *S. brevicaulis* observed by Challenger and Charlton<sup>37</sup> does not seem to be preceded by formation of a sulphonium derivative, since methionine methiodide gives dimethyl sulphide but no methanethiol in cultures of *S. brevicaulis*. This decomposition appears to be analogous to the evolution of dimethyl sulphide (but no methanethiol) from the marine alga *Polysiphonia fastigiata* observed by Haas,<sup>102</sup> as F. Challenger and (Miss) M. I. Simpson (forthcoming publication) have shown that the precursor (or a fragment of the precursor) of the dimethyl sulphide in the alga is a salt of dimethyl-2-carboxyethylsulphonium hydroxide,  $\text{Me}_2\text{S}^+(\text{OH})\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CO}_2\text{H}$  or  $\text{Me}_2\text{S}^+\text{CH}_2\cdot\text{CH}_2\cdot\text{COO}^-$ . This was isolated from *P. fastigiata* as the chloride (the bromide is already known<sup>103</sup>) and characterised as various derivatives, all of which readily evolved dimethyl sulphide at ordinary temperature in presence of sodium hydroxide. The sulphonium (thetine) salt may arise from methionine by deamination and oxidation, or from cysteine. Apart from the possible existence of a sulphonium compound in dogs' urine,<sup>31</sup> and the isolation of an oxygenated derivative of diallyl disulphide from garlic<sup>104</sup> (which may be the monosulphoxide and therefore of "sulphonium type"), this is the first recorded instance of the occurrence of a sulphonium compound in Nature.

F. C.

<sup>99</sup> *J. Biol. Chem.*, 1939, **128**, cviii; **131**, 57.

<sup>100</sup> M. A. Bennett, G. Medes, and G. Toennies, *Growth*, 1944, **8**, 59.

<sup>101</sup> G. Toennies, *J. Biol. Chem.*, 1940, **132**, 455; G. Toennies and J. Kolb, *J. Amer. Chem. Soc.*, 1945, **67**, 849.

<sup>102</sup> P. Haas, *Biochem. J.*, 1935, **29**, 1298.

<sup>103</sup> G. Carrara, *Gazzetta*, 1893, **23**, i, 506; E. I. Büllmann and K. A. Jensen, *Bull. Soc. chim.*, 1936, **3**, 2306; B. Holmberg, *Arkiv Kemi, Min. Geol.*, 1946, **21 B**, No. 7, 1.

<sup>104</sup> C. J. Cavallito and J. H. Bailey, *J. Amer. Chem. Soc.*, 1944, **66**, 1950; C. J. Cavallito, J. S. Buck, and C. M. Suter, *ibid.*, p. 1952.



## 3. STRUCTURAL PROTEINS OF MUSCLE.

The proteins of muscle may be classified into two groups: (1) those with a structural function; and (2) the soluble proteins of the sarcoplasm. Strictly defined, Group 1 embraces the extracellular types (vascular tissue, collagen, the reticulin of the sarcolemma) and also components of intracellular origin (the proteins of the myofibril and of the nuclei). Of these, only those proteins which are assumed to compose the contractile elements will be discussed. The proteins of Group 2 are largely enzymic, associated for the most part with the reactions of glycolysis, and will be discussed in a subsequent review. Some enzymes (succinic dehydrogenase, diaphorase, cytochrome oxidase) resist extraction with water and appear to be attached to the structural components.

*Proteins of the Myofibril.*

In a previous review,<sup>1</sup> some emphasis was given to the view that the fibril, by virtue of its contractile function, must consist mainly if not wholly of proteins of the polymeric fibrous type. Of the classical protein fractions, as for example those of press-juice (myogen, globulin X, myoalbumin)<sup>1,2</sup> and the globulin obtained by salt extraction, only the last, containing the myosin complex, could be assigned to the fibril. The myosin chains were considered to run in a regularly oriented manner through the anisotropic (*A*) bands, and in a less oriented fashion through the isotropic (*I*) bands. The more crystalline parts of the structure gave, both in living and in dried muscle,<sup>3,4</sup> and also in partially oriented films of isolated myosin,<sup>4,5</sup> a wide-angle X-ray pattern of the  $\alpha$ -type, indicating that the same intramolecular fold, shown later to exist in fibrinogen, fibrin,<sup>6</sup> and tropomyosin,<sup>7</sup> had been adapted for the elaboration of the ultimate contractile element. With the discovery by N. M. Liubimova and V. A. Engelhardt<sup>8</sup> in 1939 that the adenosinetriphosphatase (ATPase) activity of muscle was always associated with myosin itself, and could not by any ordinary means be separated from it, there appeared a direct link between the contractile mechanism and an energy-yielding reaction. These various studies converged to give for the first time a clue to the nature of the contractile mechanism, and on them several tentative hypotheses for the more detailed mechanism were advanced.<sup>9,10</sup> Recently, the problem has become more complicated by the

<sup>1</sup> K. Bailey, *Advances in Protein Chemistry*, 1944, **1**, 289.

<sup>2</sup> Reviewed by M. Dubuisson, *Bull. Soc. Roy. Sci. Liège*, 1945, 113.

<sup>3</sup> W. T. Astbury, Croonian Lecture, *Proc. Roy. Soc.*, 1947, *B*, in press.

<sup>4</sup> W. T. Astbury and S. Dickinson, *Nature*, 1935, **135**, 95, 765.

<sup>5</sup> *Idem*, *Proc. Roy. Soc.*, 1940, *B*, **129**, 307.

<sup>6</sup> K. Bailey, W. T. Astbury, and K. M. Rudall, *Nature*, 1943, **151**, 716.

<sup>7</sup> K. Bailey, *ibid.*, 1946, **157**, 368.      <sup>8</sup> *Biochimica*, 1939, **4**, 716.

<sup>9</sup> K. Bailey, *Biochem. J.*, 1942, **36**, 121.

<sup>10</sup> M. Dainty, A. Kleinzeller, A. S. C. Lawrence, M. Miall, J. Needham, D. M. Needham, and S.-C. Shen, *J. Gen. Physiol.*, 1944, **27**, 355; J. Needham, A. Kleinzeller, M. Miall, M. Dainty, D. M. Needham, and A. S. C. Lawrence, *Nature*, 1942, **150**, 46.

discovery of two new proteins, both occurring in the fibril, and both of asymmetric character, the actin of F. B. Straub<sup>11</sup> and tropomyosin.<sup>7</sup>

*Structure of the Myofibril.*—The level of molecular organisation observed in the electron microscope (EM) falls within the range of the larger periodicities revealed by X-rays, which in wide-angle diffraction are also used to elucidate the smaller repeating units. In an extensive examination of muscle,<sup>3</sup> both living and dried, the predominant wide-angle pattern is that of the  $\alpha$ -keratin type, which does not change after a moderate contraction. The significance of this fact has been discussed at length by Astbury, and leads, somewhat paradoxically at first sight, to the conclusion that contraction over the physiological range is not so much the transformation of the crystalline parts of the fibril from which the diffraction pattern arises as the more regular folding in series of the less crystalline parts. By the capacity of these molecules to build up intramolecular combinations, the shortening of muscle, as of myosin and keratin, involves changes of internal energy rather than of entropy.

In muscle as in other structures, the EM and X-rays confirm the presence of a large-scale pattern superimposed upon the smaller intramolecular pattern, and the comparison of patterns as between members of the keratin-myosin-fibrinogen group is of the highest importance. I. MacArthur<sup>12</sup> has shown that a correspondence exists between these larger spacings in wool, porcupine quill tip, and dried frog sartorius muscle, but it cannot yet be held that the full periods are identical, since their evaluation is not unambiguous. According to American workers<sup>13</sup> the master-period in muscle is at least 350–420 Å., whilst the probable width of the diffracting elements (27 Å.) allows of only a few polypeptide chains.

In the adductor muscles of molluscs a new type of fibril occurs, different both in its resistance to disintegration by salt solutions, and in its large-scale molecular pattern.<sup>14</sup> After maceration in 0.3M-potassium chloride and differential centrifugation, the muscle yields a fraction containing intact, needle-shaped fibrils (unfortunately designated “paramyosin”) which disintegrate in 0.45M-potassium chloride. These vary from 200 to 1000 Å. in width and 1 to 40  $\mu$  in length. With an “electron stain” they reveal a regular lattice of deeply staining spots, of separation 193 Å. perpendicular to the fibre axis, and 720 Å. parallel. The separation of *rows* of spots along the axis is, however, only one-fifth of this latter distance. X-Ray studies<sup>15</sup> had earlier indicated a master-period of 725 Å. There is no apparent change of lattice dimensions after contraction, and Schmitt *et al.*<sup>13</sup> suggest that the fibrils may serve a purely mechanical function in these rather specialised muscles. It should be noted here that the EM merely records density

<sup>11</sup> *Stud. Inst. Med. Chem. Univ. Szeged*, 1942, **2**, 3; *idem, ibid.*, 1943, **3**, 23.

<sup>12</sup> *Nature*, 1943, **152**, 38.

<sup>13</sup> R. S. Bear, *J. Amer. Chem. Soc.*, 1945, **67**, 1625; F. O. Schmitt, R. S. Bear, C. E. Hall, and M. A. Jakus, *N.Y. Acad. Sci.*, Conference on “Muscle contraction”, 1946.

<sup>14</sup> C. E. Hall, M. A. Jakus, and F. O. Schmitt, *J. Appl. Physics*, 1945, **16**, 459.

<sup>15</sup> R. S. Bear, *J. Amer. Chem. Soc.*, 1944, **66**, 2043.

(and/or thickness) gradients, and the repeating units of protein pattern deduced by X-rays should not be revealed by the EM except where they coincide with stainable material associated with the protein. Such material, as in the above lattice, may be of mineral nature, or may form part of the normal extractives (ATP, etc.) of muscle.

With an electron stain, striated muscle shows all the details elicited by histological techniques.<sup>16</sup> It shows too that on contraction there is a migration of some substance in the *A* band towards the *I*. In both *A* and *I* bands, the myosin filaments pursue an uninterrupted course, being rather less aligned in the latter, and, most strikingly, the picture remains much the same after contractions of 50%. The absence of gross change thus tends to support the intramolecular folding of chains as the mechanism of contraction, and disproves the hypothesis of A. Szent-Györgyi<sup>17</sup> based upon studies of the myosin-actin interaction, of a spiral, spring-like mechanism.

*Isolated Myosin.*—General properties have recently been reviewed<sup>1, 18</sup> and will not be described again. EM Photographs of myosin dispersed in salt solutions reveal particles derived by a random fragmentation of the fibrillar substance, varying in width (50—250 Å.) and up to 15,000 Å. in length;<sup>16, 19</sup> the average for rabbit myosin is  $120 \times 4100$  Å. Such polydispersity clearly invalidates attempts to assess particle weight by conventional methods.<sup>20</sup> It has a bearing too on the nature of Szent-Györgyi's myosin A.<sup>17, 21</sup> Since this is the fraction which yields most readily to salt extraction, it may consist of those parts of the fibril most easily fragmented and may thus comprise the shorter myosin micelles; its low viscosity tends to support this inference. The crystallinity of myosin A in the accepted sense cannot be admitted.

The earlier electrophoretic studies<sup>9, 20</sup> of myosin sols have been extended by M. Dubuisson.<sup>22</sup> Three components,  $\alpha$ ,  $\beta$ , and  $\gamma$ , in the proportions 25, 70, 5% (rabbit myosin), have been distinguished. (These electrophoretic designations must not be confused with  $\alpha$ - and  $\beta$ -configurations.) The  $\alpha$ -component carried the turbidity of the solution; the  $\gamma$  was absent from exhausted muscle and the  $\alpha$  markedly decreased. The separation of the components by fractional salting out,<sup>23</sup> combined with a study of their physical properties, suggests that the  $\alpha$ -fraction has a larger (average) particle weight than the  $\beta$ . The existence of electrophoretic components might imply (a) that myosins of differing composition and hence of differing net charge occur, (b) that various complexes of myosin with other substances (actin, tropomyosin, ATPase) exist, or (c) that the net charge is dependent to some extent upon the degree of aggregation of the molecules, i.e., upon

<sup>16</sup> C. E. Hall, M. A. Jakus, and F. O. Schmitt, *Biol. Bull. Woods Hole*, 1946, **90**, 32.

<sup>17</sup> *Acta Physiol. Scand.*, 1945, **9**, suppl. 25.

<sup>18</sup> V. A. Engelhardt, *Advances in Enzymology*, 1946, **6**, 147.

<sup>19</sup> M. V. Ardenne and H. H. Weber, *Kolloid-Z.*, 1941, **97**, 322.

<sup>20</sup> M. Ziff and D. H. Moore, *J. Biol. Chem.*, 1944, **153**, 653.

<sup>21</sup> A. Szent-Györgyi, *Stud. Inst. Med. Chem. Univ. Szeged*, 1943, **3**, 76.

<sup>22</sup> *Experientia*, 1946, **2**, 258.

<sup>23</sup> *Idem* (private communication).

the size of the micelle. In view of the known randomness of particle size, (c) is the most likely, (b) a possible, and (a) an improbable explanation.

Analytically, myosin is distinguished by its high content of free carboxyl and basic groups, and in general amino-acid composition resembles fibrinogen.<sup>1</sup> The highly charged character is admirably suited to processes requiring changes in the state of aggregation, and thus to the rôle which both proteins play in their respective biological environment. Improved values for the hydroxyamino-acids<sup>24</sup> and the bases<sup>25</sup> have been obtained, but it is probable from the careful titration data of M. Dubuisson<sup>26</sup> that the dicarboxylic acids<sup>27</sup> are underestimated. Analyses of myosin suffer from the lack of any criterion for the purity of the protein. Besides ATPase, which may or may not be identical with myosin, there are present traces of nucleic acid,<sup>28</sup> adventitious enzymes, and, for the type of preparation usually analysed, 1—2% of actin.<sup>29</sup>

*Adenosine Triphosphatase.*—All available evidence suggests that ATPase is either very firmly bound to, or part of, myosin itself. The salient properties of the enzyme, already reviewed,<sup>1,18</sup> are : (1) its specific activation by the Ca ion<sup>9</sup> and the remarkable effect of Mg<sup>++</sup> in antagonizing this action;<sup>30</sup> (2) the inability to split more than one phosphate from ATP<sup>9</sup> (if myosin is purified at a somewhat alkaline pH, it appears to retain myokinase which carries the degradation to adenylic acid<sup>31</sup>); (3) the alkaline pH optimum of 9;<sup>8,9</sup> (4) the protective action of amino-acids<sup>9</sup> (and carnosine) against heavy metal inhibition; (5) the sulphhydryl character of the enzyme.<sup>32</sup> SH oxidants (porphyrindin<sup>32</sup> hydrogen peroxide<sup>33</sup>), thiol reagents (*p*-chloro-mercuribenzoate<sup>32</sup>) or alkylating reagents (chloroacetophenone, iodoacetate<sup>34</sup>) all reduce or destroy ATPase activity. However, oxidants are more effective inhibitors than alkylating reagents,<sup>32,34</sup> and these must be added in greater concentration than is necessary for most accredited SH enzymes. In considering the evidence for the identity of ATPase and myosin, it is noteworthy that the extent of reaction of an oxidant such as iodosobenzoate with myosin SH groups<sup>46</sup> is also greater than that of a powerful alkylating reagent such as chloroacetophenone.<sup>34</sup> In these respects, enzyme properties run parallel with those of myosin itself. (Another peculiarity of the SH groups of myosin was observed by W. C. Hess and M. X. Sullivan.<sup>35</sup> Hydrolysis of a myosin sol yields about 1% of the protein weight as cysteine, but hydrolysis of myosin dried in a vacuum yields entirely cysteine.)

<sup>24</sup> M. W. Rees, *Biochem. J.*, 1946, **40**, 632.

<sup>25</sup> H. T. Macpherson, *ibid.*, p. 470.

<sup>26</sup> *Arch. Int. Physiol.*, 1941, **51**, 133; *idem, ibid.*, 1943, **53**, 308.

<sup>27</sup> J. G. Sharp, *Biochem. J.*, 1939, **33**, 679.

<sup>28</sup> K. Bailey, unpublished.

<sup>29</sup> K. Bailey and S. V. Perry, unpublished.

<sup>30</sup> G. D. Greville and H. Lehmann, *Nature*, 1943, **152**, 81.

<sup>31</sup> H. O. Singher and A. Meister, *J. Biol. Chem.*, 1945, **159**, 491.

<sup>32</sup> T. P. Singer and E. S. G. Barron, *Proc. Soc. Exp. Biol. Med.*, 1944, **56**, 120.

<sup>33</sup> J. W. Mehl, *Science*, 1944, **99**, 518; M. Ziff, *J. Biol. Chem.*, 1944, **153**, 25.

<sup>34</sup> K. Bailey, unpublished.

<sup>35</sup> *J. Biol. Chem.*, 1943, **151**, 635.

D. B. Polis and O. Meyerhof<sup>36</sup> have briefly described a method of obtaining a myosin fraction 2—3 times as active as the original. Somewhat earlier, W. H. Price and C. F. Cori<sup>37</sup> reported the separation of ATPase from myosin, and found that the enzyme was no longer activated by  $\text{Ca}^{++}$ , but was so by creatine. The claim of separation has now been withdrawn,<sup>38</sup> since further work shows that the myosin-free enzyme is creatine phosphokinase, derived from impurities in the myosin preparation.

*Actin and Myosin A.*—The many papers of Szent-Györgyi and his school<sup>17,39</sup> concerning the interaction of actin and myosin, and the hypothetical rôle of actomyosin in muscle contraction can be described only in outline. When minced muscle is left in contact with a salt solution adequate to extract myosin, the resulting *brei* gradually thickens to a gel-like consistency. This change can be simulated with the isolated components of the reaction, first by obtaining myosin A,<sup>40,41</sup> which yields to salt solutions after a 20 minute extraction period, and secondly by washing the muscle residue with an alkaline buffer, drying the residue in acetone, and extracting with water to obtain "actin".<sup>11</sup> The aqueous extract of actin is not viscous until salt is added; it then changes to a limpid gel of "active actin" which is both thixotropic and flow-birefringent. In salt solutions of ionic strength 0.5—1.5, the addition of actin to myosin A greatly increases the viscosity above that of either component at the same dilution, and this increase is nullified by addition of ATP (1 mole/70,000 g. myosin).<sup>17</sup> The action of ATP is not entirely specific, since inorganic pyrophosphate<sup>42</sup> (at 0° but not at 20°) and 5% urea<sup>43</sup> act similarly. In the EM, actomyosin appears to consist of a network of anastomosing filaments,<sup>44</sup> the type of structure which might readily be predicted from a consideration of its gel-like properties.

Myosin prepared in the classical manner differs from myosin A in containing 1—2% of actin which enhances its viscosity. The addition of ATP therefore effects a slight reduction in viscosity, an effect which was first discovered by J. Needham and his collaborators<sup>10</sup> before the discovery of actin itself. By the ATP-viscosity test, myosin A contains no, or only a trace of, actin.

In a sparse ionic atmosphere, the interaction of actin, myosin, ATP and salt ions leads to interesting effects which have been woven rather prematurely into a theory of muscle contraction.<sup>17</sup> An aqueous gel of actomyosin, within certain limits of salt (potassium chloride) concentration, precipitates, and the zone of precipitation is narrowed in presence of Mg ions and/or ATP. In addition, ATP causes an enhanced shrinkage of the

<sup>36</sup> *J. Biol. Chem.*, 1946, **163**, 339.

<sup>37</sup> *Ibid.*, **162**, 393.

<sup>38</sup> C. F. Cori, *ibid.*, **165**, 395.

<sup>39</sup> S. Karges, "Studies from the Institute of Medical Chemistry University Szeged", Basle and New York, 1941—1942, **1**; 1942, **2**; 1943, **3**.

<sup>40</sup> I. Banga and A. Szent-Györgyi, *ibid.*, 1941—1942, **1**, 5.

<sup>41</sup> A. Szent-Györgyi, *ibid.*, 1943, **3**, 76.

<sup>42</sup> F. B. Straub, *ibid.*, 1943, **3**, 38.

<sup>43</sup> W. F. H. M. Mommaerts, *Arkiv. Kemi, Min. Geol.*, 1945, **19A**.

<sup>44</sup> W. T. Astbury, S. V. Perry, and R. Reed (private communication).

particles, and this effect has been studied in some detail with threads of actomyosin, prepared by dissolving the complex in 0.5M-potassium chloride and squirting the solution into 0.05M-potassium chloride. If the environment is now changed to one consisting of 0.1M-potassium chloride-0.01M-magnesium chloride-0.09% sodium-ATP, an isodimensional contraction of 60% is produced in 5 minutes. As Astbury<sup>3</sup> has pointed out, this synaeresis of actomyosin in presence of small concentrations of ions cannot be considered unique in chain-molecular systems. Its most important feature is the enhancing action of ATP, and the explanation of this effect must be sought in the same terms as that producing a reduction in viscosity when ATP is added to actomyosin in the stronger (0.5M) salt solutions.

Though it has not been emphasised by the Hungarian workers, the unique feature of the interaction of myosin and actin is that it occurs at ionic strengths<sup>17,45</sup> (up to 2M) which would greatly reduce the purely electrostatic interaction of one protein with another. It seems likely then that a special interaction is involved, perhaps a type of co-ordination, in which the actin and myosin interact at some specific chemical grouping. This inference was fruitful, since it led to the finding<sup>46</sup> that SH reagents (iodoacetate, iodoacetamide, *p*-chloromercuribenzoate, *o*-iodosobenzoate) prevented the interaction. Only the SH groups of myosin are concerned: actin itself is rich in SH groups, but, of these, 0.8% (as cysteine/100 g. protein) may be oxidized by iodosobenzoate without influencing appreciably the reaction with myosin. By contrast, the oxidation of the cysteine of myosin to the extent of 0.5% (total 1.16%) prevents actomyosin formation. Moreover, the concentrations of the various poisons (as m-mol./mg. myosin) which inhibit actomyosin formation are almost identical with those which Singer and Barron<sup>32</sup> found to inhibit ATPase. This quantitative correlation between myosin SH groups and its ATPase activity on the one hand, and what might be termed its gross colloidal behaviour on the other, argues strongly for the identity of myosin and ATPase; particularly so, when the substrate for the enzyme reaction (ATP) so profoundly influences the colloidal reaction. These interrelationships are further strengthened by the fact that ATP, wherever it acts as substrate, does so with enzymes either of proven or suggested SH character (creatine phosphokinase,<sup>47</sup> yeast hexokinase,<sup>48</sup> the choline acetylase system<sup>49</sup>), and may be deemed to have an affinity for some type of SH grouping to be found in proteins.

In the light of these facts, it is supposed that certain SH groupings in myosin, probably identical with those of ATPase, can interact either with actin (through an unknown group) or with ATP, but that ATP competes more successfully, and transforms the actomyosin gel into its freely-moving

<sup>45</sup> F. Guba, *Stud. Inst. Med. Chem. Univ. Szeged*, 1943, **3**, 40.

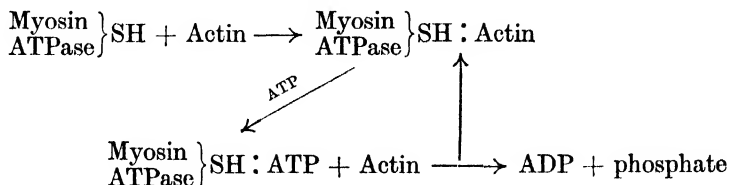
<sup>46</sup> K. Bailey and S. V. Perry, *Proc. Biochem. Soc.*, 1947, **41**, in press.

<sup>47</sup> H. Lehmann and L. Pollak, *Biochem. J.*, 1942, **36**, 672.

<sup>48</sup> R. van Heyningen, Report to Ministry of Supply, by M. Dixon, 1942, No. 10; K. Bailey and E. C. Webb, *ibid.*, 1944, No. 30.

<sup>49</sup> D. Nachmansohn and H. M. John, *J. Biol. Chem.*, 1945, **158**, 157.

components. In concentrated salt solution the effect is revealed in viscosity reduction; in very dilute salt solutions, the loss of gel structure allows the myosin particles to precipitate in the way that actin-free, salt-free gels of myosin precipitate on addition of small amounts of salt. Schematically :



The view that the ATP-myosin-actin interaction is the keystone of muscle contraction is quite premature until more is known of the nature of actin, the nature of the forces involved in its interaction, and the nature of the groups we have termed "sulphydryl" but which possess properties not readily explained as simple reactions of the ordinary thiol group.<sup>18, 50</sup> The plausibility of a hypothesis must not be mistaken as its proof, and the invocation of the lock and key mechanism, whereby actin is the lock and ATP the key,<sup>17</sup> must obviously be explored; but the scope for hypothesis in the explanation of muscle contraction is so great, and the possibilities so numerous, that it is more profitable to dissect the pieces than to construct the whole. Any ultimate interpretation must show how ATP and actin affect the intramolecular contractility of myosin chains; it would not seem to involve a consideration of synaeresis effects.

*Tropomyosin.*—This newly discovered protein<sup>7</sup> of the fibril is of asymmetric character but of relatively low molecular weight (about 90,000). In rabbit skeletal muscle it comprises 0.5% of the fresh muscle weight. Though water-soluble after isolation, it cannot be extracted from minced muscle by water and only slowly by salt solutions. Likewise, it is not extracted by water from washed muscle residue dried in ethanol-ether, but is so by M-potassium chloride. These properties suggest a metathetic link with one or more constituents of the fibril.

In salt-free solutions, tropomyosin is extremely viscous and shows positive flow-birefringence; addition of salt to 0.1M effects a large reduction in viscosity (the reverse of the effect of salts on actin), and such solutions when subjected to isoelectric crystallisation procedures<sup>51</sup> deposit large birefringent plates containing 90% of water. EM Studies<sup>52</sup> show that the enhanced viscosity in absence of salt is due to the perfectly regular aggregation of particles into fibres, built up presumably by electrostatic interaction of one molecule with another. The phenomenon might suggest the mechanism whereby the polymeric proteins (keratin, collagen, myosin) are *initially* elaborated from smaller units. The depolymerising action of guanidine and

<sup>50</sup> Review by H. Neurath, J. P. Greenstein, F. W. Putnam, and J. O. Erickson, *Chem. Reviews*, 1944, **34**, 157.

<sup>51</sup> K. Bailey, unpublished.

<sup>52</sup> W. T. Astbury and R. Reed, private communication.

urea gives some clue to the size of the submolecules which make up the native protein, since it is unlikely that anything more than a splitting of hydrogen bonds is involved.<sup>53</sup> In urea, myosin does in fact depolymerise to units of the same average molecular weight<sup>54</sup> (100,000) as tropomyosin.

The significance of tropomyosin rests entirely in the possibility that it is a sub-unit of myosin. Not only is it an  $\alpha$ -protein *par excellence*, but the amino-acid composition, now completed, is entirely of myosin type. The two analyses are not identical, since tropomyosin is rather more polar, and in any case we cannot consider that a pure myosin has yet been analysed or that myosin as we know it has been adequately analysed. The evidence for some fundamental relation between the two proteins, from structure, analysis, occurrence in the same histological site, is so impressive that the name tropomyosin has been adopted to suggest it. Its existence as an  $\alpha$ -keratin type which is both fibrous and crystalline is a logical outcome of all that is implied in the systematic researches of the Leeds school.

K. B.

#### 4. MAGNETIC PROPERTIES OF HÆMATIN DERIVATIVES.

*Magnetic Susceptibility.*—The volume susceptibility  $\kappa$  of a substance is the ratio of the intensity of magnetisation to the strength of the magnetic field:  $\kappa = I/H$ , and the mass susceptibility (*i.e.*, per unit mass)  $\chi = \kappa/\rho$ . Apart from the few ferromagnetics, all substances may be classified as diamagnetic ( $\chi$  negative) or paramagnetic ( $\chi$  positive). In a non-uniform magnetic field these two groups are subjected to forces directing them away from or towards the region of maximum  $H$  respectively. Diamagnetism is a property of all matter arising from the effect of the field on the orbital motion of the electrons and has been recently reviewed.<sup>1</sup> Certain substances (*e.g.*, salts of transition elements, or oxygen) as well as organic free radicals<sup>2</sup> possess a permanent magnetic moment arising from unpaired electron spins and therefore exhibit a pronounced paramagnetism which swamps the numerically much smaller diamagnetism.

Curie showed that paramagnetism usually obeyed the law

$$\chi_m = C_m/T \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where  $\chi_m = \chi M$  and  $C_m$  = the Curie constant per g.-mol. The classical theory of Langevin for paramagnetic gases derives an expression  $\chi_m = \sigma_0^2/3RT$  where  $\sigma_0$  (g.-mol. magnetic moment) =  $\mu$  (molecular magnetic moment)  $\times N$ . From the Bohr theory of atomic structure the natural quantum unit of magnetic moment =  $9.174 \times 10^{-21}$  E.M.U. Hence unit per g.-mol. =  $9.174N \times 10^{-21} = 5564$  E.M.U. This quantity is known as the *Bohr magneton* ( $\mu_B$ ). Hence

$$\mu_B = \frac{\sigma_0}{5564} = \frac{\sqrt{3RC_m}}{5564}.$$

<sup>53</sup> A. E. Mirsky and L. Pauling, *Proc. Nat. Acad. Sci.*, 1936, **22**, 439.

<sup>54</sup> H. H. Weber and R. Stöver, *Biochem. Z.*, 1933, **259**, 269.

<sup>1</sup> W. R. Angus, *Ann. Reports*, 1941, **38**, 27.     <sup>2</sup> D. H. Hey, *ibid.*, 1940, **37**, 263.



Substitution from (1) gives

$$\mu_B = 2.84\sqrt{T\chi_m} \quad (2)$$

From the quantum-mechanical development of Langevin's theory equations are derived relating  $\mu_B$  to the number of unpaired electron spins. The simplest type is in the form

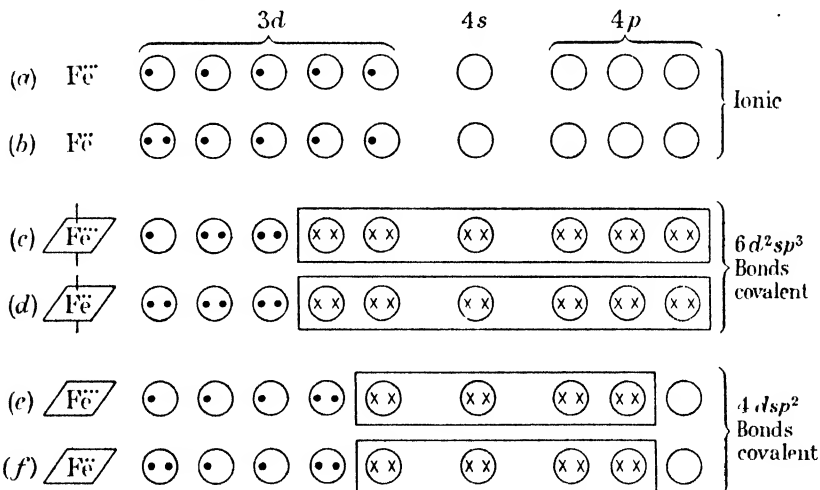
$$\mu_B = g\sqrt{j(j+1)} \quad (3)$$

where  $g$  and  $j$  are functions of the orbital and spin moments of the electrons. This formula has successfully been applied to the paramagnetic rare earths,<sup>3</sup> where the unpaired electrons are in an inner shell and consequently shielded from the influence of neighbouring molecules. When considering salts of iron and other transition elements it is necessary to postulate a considerable diminution or even the disappearance of the orbital moment in order to account for the experimental figures. Equation (3) now reduces to

$$\mu_B = 2\sqrt{s(s+1)} \quad (4)$$

where  $s$  = resultant electron spin moment of the atom. The loss of orbital contribution is ascribed to the close proximity of other molecules in the liquid and solid states.<sup>4</sup>

**Electronic Structure and Magnetic Moment.**—The  $\text{Fe}^{+++}$  and  $\text{Fe}^{++}$  ions contain 24 and 25 orbital electrons respectively. Omitting the inner 18, which make up the stable argon configuration of 9 paired electrons, the arrangement of the remainder in the  $3d$  shell can be expressed by (a) and (b) with 5 and 4 unpaired electrons (u.e.), respectively:



<sup>3</sup> H. Terrey and O. J. Walker, *Ann. Reports*, 1937, **34**, 122.

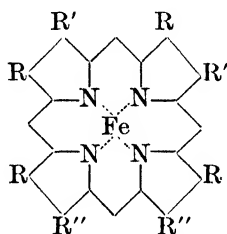
<sup>4</sup> For full discussion of theoretical aspects reference should be made to E. C. Stoner, "Magnetism and Matter" (Methuen, 1934), and to J. H. Van Vleck, "The Theory of Electric and Magnetic Susceptibilities" (Oxford, 1932). Less advanced treatments and also experimental methods are given by S. S. Bhatnagar and K. N. Mathur, "Physical Principles and Applications of Magnetochemistry" (Macmillan, 1935),

In complex salts such as ferri- and ferro-cyanides two electrons from each  $\text{CN}'$  (Sidgwick's lone pairs)<sup>5</sup> fill the outer orbitals, leading to the stable krypton configuration and to a decrease in paramagnetism following the pairing of  $3d$  electrons, ( $c$ ) and ( $d$ ). The structure of square 4-covalent complexes can be expressed by ( $e$ ) and ( $f$ ), but in such cases the maximum co-ordination number of 6 may be achieved by the formation of two ionic bonds when the probable structure is a resonance equilibrium of six equivalent bonds of intermediate type.<sup>6</sup> No examples of type ( $f$ ) are known,\* and ( $e$ ) is limited to a few hæmatin derivatives.

The value of  $s$  in equation (4) is  $\frac{1}{2}$  for each unpaired electron; hence, from (2) and (4) the relationships between the number of u.e. and the paramagnetism and valency of the iron may be calculated (Table I). As L. Cambi and L. Szegoe<sup>7</sup> have shown that hæmin obeys Curie's law, these figures can be applied to its derivatives.

TABLE I.

Unpaired electrons .....	0	1	2	3	4	5
$\mu_B$ .....	0	1.73	2.83	3.87	4.90	5.92
$10^6 \chi_m$ (20°) .....	0	1270	3390	6350	10,180	14,820
Valency of Fe .....	2	3	2	3	2	3



$R = \text{Me.}$

$R' = -\text{CH}:\text{CH}_2.$

$R'' = -\text{CH}_2\cdot\text{CH}_2\cdot\text{CO}_2\text{H.}$

*Hæm* (ferrous protoporphyrin) : the active group of hæmoglobin. Full lines represent bonds which, on account of resonance, are intermediate between single and double bonds.

*Structure and Nomenclature of Hæmin Derivatives.*—The interrelationships of hæmatin derivatives have been formulated by D. Keilin<sup>9</sup> but the introduction of a new system of nomenclature<sup>10</sup> has led to some confusion.

and by P. W. Selwood, "Magnetochemistry" (Interscience Publishers, 1943). Experimental technique (Gouy method) is described by C. M. French and V. C. G. Trew, *Trans. Faraday Soc.*, 1945, **41**, 439, and by L. Pauling and C. D. Coryell, *Proc. Nat. Acad. Sci.*, 1936, **22**, 159.

<sup>5</sup> "Electronic Theory of Valency" (Oxford, 1937).

<sup>6</sup> L. Pauling, "The Nature of the Chemical Bond" (Cornell University Press, 1942).

<sup>7</sup> *Rend. Ist. Lombardo sci.*, 1934, **67**, 275.

<sup>8</sup> M. L. Huggins, *Ann. Rev. Biochem.*, 1942, **11**, 552.

<sup>9</sup> *Ergebn. Enzymforsch.*, 1933, **2**, 239.

<sup>10</sup> L. Pauling and C. D. Coryell, *Proc. Nat. Acad. Sci.*, 1936, **22**, 210.

\* With the possible exception of ferrous phthalocyanine (*J. pr. Chem.*, 1939, **154**, 73).

The two systems are summarised in Table II, where the basic structures of the more important derivatives are given. In this report the original nomenclature will be used.

TABLE II.

Original nomenclature.	Valency of Fe.	Groups attached to Fe other than porphyrin.	New nomenclature.
Hæm *	2	( $2\text{H}_2\text{O} ?$ ) †	Ferroheme
Hæmin	3	( $\text{Cl}^-$ )	{ Hemin
Hæmatin	3	$\text{OH}^-$ ( $\text{H}_2\text{O} ?$ ) †	{ Ferriheme chloride
Hæmoglobin * (Hb)	2	globin	Ferriheme hydroxide
Oxyhæmoglobin	2	$\text{O}_2$ globin	Ferrohemoglobin
Carbon monoxide Hb	2	$\text{CO}$ globin	Oxyhemoglobin
Acid methæmoglobin	3	globin ( $\text{H}_2\text{O} ?$ )	Carbonmonoxy Hb
Alkaline metHb	3	globin $\text{OH}^-$	Ferrihemoglobin
Hæmochromogen *	2	{ denatured globin or 2 mols. { organic base (e.g., pyridine)	Ferri Hb hydroxide
Parahæmatin	3		{ Ferrohemochromogen
			{ Ferrihemochromogen

\* These combine reversibly with  $\text{CO}$ .

† See structures postulated by T. H. Davies, *J. Biol. Chem.*, 1940, **135**, 597.

*Magnetic Measurements on Hæmatin Derivatives.*—Work in this field up to 1941 has been summarised by Selwood<sup>4</sup> and by D. L. Drabkin.<sup>11</sup> The more recent publications have been devoted to catalase and peroxidase. The susceptibilities of the derivatives under review are collected in Table III.

(A) *Hæmoglobin and its (ferrous) derivatives.* The first precise magnetic measurements on hæmoglobin (Hb) and on  $\text{HbO}_2$  and  $\text{HbCO}$  were made by Pauling and Coryell.<sup>10</sup> The iron of the oxygen and the carbon monoxide derivatives has zero magnetic moment, while the paramagnetism of Hb corresponds to  $\mu_B = 5.46$ , which is in excess of the theoretical 4.90 for 4 u.e. The high value was attributed to hæm-hæm interactions which tend "to stabilise to some extent the parallel configuration of the moments of the four hemes in the molecule." The alternative explanation of an appreciable orbital contribution was rejected through consideration of certain ferrous complexes containing nitrogen.<sup>12</sup> The combination of two paramagnetics, Hb and oxygen, to give a compound with zero moment must result in a profound change in the oxygen molecule involving the disappearance of two u.e. Electronic structures for  $\text{HbO}_2$  and  $\text{HbCO}$  are put forward. Compounds of Hb with ethyl isocyanide<sup>13</sup> and with cyanide ion and nitric oxide<sup>14</sup> have zero moment. Thus in Hb the iron bonding is ionic, while in the derivatives the iron is covalently linked.

By taking Hb ( $\mu_B = 5.43$ ) and  $\text{HbCO}$  ( $\mu_B = 0$ ) as standards at  $24^\circ$ , C. D. Coryell, F. Stitt, and L. Pauling<sup>15</sup> devised a simple method for determining  $\chi_m$  and  $\mu_B$  for derivatives of Hb, using the Gouy technique. If  $\Delta_{w\text{Hb}}$  is the apparent change in weight on applying the magnetic field to a tube of  $\text{HbO}_2 +$  reducing agent ( $\text{Na}_2\text{S}_2\text{O}_4$ ) and  $\Delta_{w\text{HbCO}}$  the corresponding change after

<sup>11</sup> *Ann. Rev. Biochem.*, 1942, **11**, 552.

<sup>12</sup> L. Pauling, *J. Amer. Chem. Soc.*, 1931, **53**, 1367.

<sup>13</sup> C. D. Russell and L. Pauling, *Proc. Nat. Acad. Sci.*, 1939, **25**, 517.

<sup>14</sup> F. Stitt and C. D. Coryell, *J. Amer. Chem. Soc.*, 1939, **61**, 1263.

<sup>15</sup> *Ibid.*, 1937, **59**, 633.

saturation with carbon monoxide, then  $\Delta_{w\text{Hb}} - \Delta_{w\text{HbCO}}$  is a measure of the paramagnetism of Hb ( $\chi_m = 12,290 \times 10^{-6}$ ) after correction for the diamagnetism of the  $\text{Na}_2\text{S}_2\text{O}_4$ . If  $\Delta_w$  is the observed change in weight with an equimolecular solution of a Hb derivative, the molar susceptibility and magnetic moment of the latter can be obtained from

$$\chi_m = \frac{\Delta_w - \Delta_{w\text{HbCO}}}{\Delta_{w\text{Hb}} - \Delta_{w\text{HbCO}}} \cdot 12,290 \times 10^{-6} \quad \mu_B = \left( \frac{\Delta_w - \Delta_{w\text{HbCO}}}{\Delta_{w\text{Hb}} - \Delta_{w\text{HbCO}}} \right)^{\frac{1}{2}} \cdot 5.43$$

The figures  $12,290 \times 10^{-6}$  and 5.43, which are due to C. D. Coryell and F. Stitt,<sup>16</sup> are slightly lower than the original figures of Coryell *et al.*<sup>15</sup>

D. S. Taylor and C. D. Coryell<sup>17</sup> found significant variations among several Hb's:

	Cow.	Horse.	Sheep.	Human.
$10^6 \chi_m$ .....	12,290	12,260	12,390	11,910
$\mu_B$ .....	$5.435 \pm 0.015$	5.43	5.46	5.35

The differences were ascribed to variations in hæm-hæm interaction which are apparent also from variations in the oxygen affinity in the different species. The identical susceptibilities of laked and unlaked red blood cells are further evidence for the identity of intracorpuseular and free Hb.<sup>18</sup> Estimates of the susceptibility of the non-hæmin Fe of blood were made.<sup>19</sup>

The two theoretical bases of the Hb +  $\text{O}_2$  equilibrium proposed by G. S. Adair<sup>20</sup> and by L. Pauling<sup>21</sup> have been discussed by C. D. Coryell, L. Pauling, and R. W. Dodson<sup>22</sup> from the magnetic standpoint. They conclude that the susceptibilities are more in accord with Pauling's view of four essentially independent hæms in the Hb molecule where the oxygen affinity of one hæm is influenced by the oxygenation of a neighbouring hæm. Adair's concept of a 4-fold hæm structure combining progressively with 1-4 oxygen molecules appears to require a much higher value for the magnetic moment. The theory of Hb structure due to J. Wyman<sup>23</sup> postulates that the two dissociable acid groups of Hb detected by electrode-potential measurements within the range pH 5-9 are iminazole groups of histidine by which Fe is linked to the protein.<sup>24</sup> C. D. Coryell and L. Pauling,<sup>25</sup> from a consideration of potentiometric and magnetic data, provide a theoretical basis of the Bohr effect (variation of oxygen affinity with pH) and also of the change on oxygenation from ionic to covalent bonding in terms of resonance equilibria of the iminazole groups.

(B) *Methæmoglobin and its derivatives.* Coryell, Stitt, and Pauling<sup>15</sup> have measured the susceptibility of the acid and alkaline forms of MetHb and of the F', CN', and SH' derivatives. Their results indicate 5 and 3 u.e.

<sup>16</sup> *J. Amer. Chem. Soc.*, 1940, **62**, 2942. <sup>17</sup> *Ibid.*, 1938, **60**, 1177.

<sup>18</sup> D. Keilin and E. F. Hartree, *Nature*, 1941, **148**, 75.

<sup>19</sup> G. Barkan and O. Schales, *Z. physiol. Chem.*, 1937, **248**, 96.

<sup>20</sup> *Proc. Roy. Soc.*, 1925, **A**, **109**, 299.

<sup>21</sup> *Proc. Nat. Acad. Sci.*, 1935, **21**, 186.

<sup>22</sup> *J. Physical Chem.*, 1939, **43**, 825.

<sup>23</sup> *J. Biol. Chem.*, 1939, **127**, 581.

<sup>24</sup> The iminazole theory has been criticised by H. F. Holden, *Ann. Rev. Biochem.*, 1945, **14**, 599.

<sup>25</sup> *J. Biol. Chem.*, 1940, **132**, 769.

for acid and alkaline MetHb respectively, 5 u.e. for the F', and 1 u.e. for the other derivatives. The considerable deviations from the theoretical figures are discussed from the points of view of hæm-hæm interactions and orbital contributions. These authors were obliged to postulate a hæm-hæm interaction as the cause of low values of magnetic moment in spite of the fact that interaction had been considered responsible for the high values found for Hb. The whole position becomes less tenable following the magnetic measurements on myoglobin (see below). Magnetic studies of the change from acid to alkaline MetHb indicated a  $pK$  of 8.12 for the equilibrium  $\text{MetHbOH} \rightleftharpoons \text{MetHb}^+ + \text{OH}'$ , and the 1 : 1 ratio of Fe to CN' in MetHbCN was confirmed. The unstable iminazole<sup>13</sup> derivative as well as compounds with azide ion and ammonia likewise appear to be essentially covalent (1 u.e.). A compound with EtOH has been reported with a moment of 5.39.<sup>16</sup> A slight variation in  $\mu_B$ , indicating three forms of acid MetHb, has been reported<sup>26</sup> corresponding to the dissociation of acid groups. The possible structures of MetHb and derivatives are discussed but without definite conclusions.

(C) *Myoglobin*. Myoglobin contains only one hæm group per molecule, hence the difference between the moments of this pigment and of Hb should be a measure of hæm-hæm interactions in the latter. D. S. Taylor<sup>27</sup> found  $\mu_B = 5.46$  and 5.85 for myoglobin and acid metmyoglobin, respectively, which are virtually identical with the corresponding Hb figures. The excess over 4.90 in the case of Hb and myoglobin must therefore be due to an exceptionally large orbital contribution. Among Fe<sup>2+</sup> salts this contribution is small (0.2—0.3) and Taylor suggests that in Fe<sup>2+</sup>-porphyrins the nitrogen atoms, being part of a rigid cyclic structure, are held at a greater distance from the Fe atom than are the anions in simple Fe<sup>2+</sup> salts. On this hypothesis a less effective quenching of the orbital contribution can be expected. The view of Pauling *et al.*<sup>22</sup> that hæm-hæm interaction can markedly modify the magnetic moment cannot be generally accepted.

(D) *Hæmin, hæmatin, hæm, and hæmochromogens*. A thorough study of these simple derivatives is an essential prerequisite for a further interpretation of the susceptibilities of the natural hæm pigments. Cambi and Szegoe<sup>7</sup> found that the paramagnetism of a pyridine solution of hæmin decreased with time. The recorded values of  $\mu_B$  for crystalline hæmin are 5.81,<sup>7</sup> 5.83,<sup>28</sup> 5.69, 5.93,<sup>29</sup> 5.77,<sup>30</sup> and 5.96—6.00. Leaving aside the last figures (calculated from the results of F. Haurowitz and B. Kittel<sup>31</sup>), the average of 5.81 indicates ionic bonds. According to Pauling and Coryell<sup>29</sup> the susceptibilities of hæmatin, hæm, and hæmochromogens indicate 5, 4, and 0 u.e., respectively. W. A. Rawlinson<sup>32</sup> investigated the same deriv-

<sup>26</sup> R. v. Zeyneck, *Z. physiol. Chem.*, 1901, **33**, 426.

<sup>27</sup> *J. Amer. Chem. Soc.*, 1939, **61**, 2150.

<sup>28</sup> Reporter's unpublished results.

<sup>29</sup> L. Pauling and C. D. Coryell, *Proc. Nat. Acad. Sci.*, 1936, **22**, 159.

<sup>30</sup> W. A. Rawlinson and P. B. Scutt, private communication.

<sup>31</sup> *Ber.*, 1933, **66**, 1046.

<sup>32</sup> *Austr. J. Exp. Biol. Med.*, 1940, **18**, 185.

atives under different conditions. He confirmed the fall of susceptibility with time of hæmin in pyridine and ascribed it to parahæmatin formation in presence of traces of water; in absence of water pyridine does not co-ordinate with hæmin. Pyridine parahæmatin (hæmin in pyridine and sodium hydroxide) has the expected covalent bonding ( $\mu_B = 1.97$ ). Pauling and Coryell<sup>29</sup> found  $\mu_B = 5.56$  for hæmatin solution (hæmin in sodium hydroxide solution) to which sucrose had been added to prevent aggregation and precipitation. In absence of sugar the lower values 3.52<sup>32</sup> and 3.23<sup>28</sup> corresponding to 3 u.e. have been obtained. The high degree of aggregation of hæmin in aqueous alkali<sup>11</sup> may give rise to these lower values. The aggregates can be broken down by addition of cyanide and consequent saturation of the Fe valencies,<sup>33</sup> and apparently also by sucrose. A measure of the influence of aqueous solvents can be obtained from the magnetic measurements of Rawlinson and Scutt<sup>30</sup> on a series of compounds in the solid state: chloro-, bromo-, acetoxy-, formoxy-, and aza-hæmins, hæmin dimethyl ester, hæmatin, and the anhydride and half anhydride of hæmatin. The experimental figures for  $\mu_B$  range between 5.71 and 5.89 except for one sample of hæmatin where the average value is 5.43. The low magnetic moment for hæmatin solutions therefore appears to be due to the associating effect of the solvent.

(E) *Cytochrome-c*. Of the components of cytochrome, only *c* can be extracted in a pure form. H. Theorell<sup>34</sup> studied the absorption spectra and the magnetic properties of the oxidised (Fe<sup>+++</sup>) pigment at varying pH and demonstrated the existence of 5 forms I—V in which  $10^6\chi_m$  ranges from 13,060 at pH 0.8 to 1900 at pH 13.5. The results are interpreted in the light of the iminazole linkage theory.<sup>23</sup> Thus type I which exists in very acid solutions resembles spectroscopically the free hæmatin of cytochrome *c* and shows 5 u.e. Type V on the other hand is a typical parahæmatin with covalent bonding. The intermediate forms represent stages in the progressive titration of the iminazole groups which consequent changes in bond type. Type III exists over the pH range 4—10 and is thus the only one of physiological significance ( $10^6\chi_m = 3300$ ). The strong covalent bonding precludes the formation of cyanide and fluoride derivatives which can be detected only at high or low pH when the Fe bonds may be loosened. A compound with nitric oxide in neutral solution has, however, been reported.<sup>35</sup> Ferrous cytochrome-*c* has the same absorption spectrum and zero magnetic moment at all pH's. It is a typical hæmochromogen except that it is not autoxidisable at physiological pH. Some loosening of the bonds must occur at extremes of pH in order to account for the observations<sup>35, 36</sup> that the pigment is autoxidisable at pH <4 and >10 and that it combines with carbon monoxide at pH 13. Theorell concludes that the essential difference in structure between Hb and cytochrome-*c* is that

<sup>33</sup> K. Zeile and F. Reuter, *Z. physiol. Chem.*, 1933, **221**, 101.

<sup>34</sup> *J. Amer. Chem. Soc.*, 1941, **63**, 1804, 1812, 1818, 1820.

<sup>35</sup> D. Keilin and E. F. Hartree, *Proc. Roy. Soc.*, 1937, *B*, **122**, 298.

<sup>36</sup> D. Keilin, *ibid.*, 1930, *B*, **106**, 418.

in the former only one of the two iminazoles is favourably orientated for strong co-ordination with iron, but in the latter two strong bonds are formed. Thus, the bond available in Hb for reaction with oxygen or carbon monoxide is only available to cytochrome-c at extremes of pH.

(F) *Catalase and peroxidase.* In order to deal with the very small quantities of these enzymes which can be obtained in the pure state, H. Theorell<sup>37</sup> constructed an apparatus for micro-determination of susceptibility. A narrow glass tube divided by a central septum into two equal lengths is suspended horizontally from two long fibres. Solvent and enzyme solution are placed in the two halves of the tube and a strong magnetic field is applied at the region of the septum. From the longitudinal displacement the paramagnetism of the iron may be calculated.

Using crystalline horse-liver catalase, H. Theorell and K. Agner<sup>38</sup> corrected the earlier figure of  $\mu_B = 4.64$ <sup>39</sup> and studied several derivatives of catalase. The magnetic study of this substance presents considerable difficulties. For instance, as the iron content is only 0.093%, very concentrated solutions must be used, involving large corrections for diamagnetism. Furthermore, in "pure" crystalline liver catalase only about 75% of the iron is present as hæmatin, the remainder being in the form of a bile-pigment derivative. The necessity of assuming a value for the susceptibility of the latter, and at the same time assuming that it constitutes 25% of the iron, introduces uncertainties into the calculations of hæmatin-Fe susceptibility. The partition of iron between hæmatin and bile pigment in pure catalase is variable,<sup>40</sup> although Theorell finds evidence for about 25% of the latter in his samples by magnetic titration with hydrogen cyanide. By analogy with acid MetHb, the iron of catalase has 5 u.e. ( $\mu_B = 5.89$ ) and hence ionic bonding. Similar bonding in azide catalase is in striking contrast to the azide derivative of MetHb. D. Keilin and E. F. Hartree<sup>41</sup> showed that azide catalase reacts with peroxides to give a derivative which combines with carbon monoxide and is autoxidisable and therefore contains ferrous iron. It was proposed by analogy that free catalase would undergo a similar cyclic valency change during the decomposition of hydrogen peroxide. According to Theorell and Agner, however, the susceptibility of azide-catalase + peroxide in nitrogen or carbon monoxide indicates that no reduction takes place. These results are criticised by Keilin and Hartree on the grounds that the peroxide derivatives are too unstable to remain unchanged during the magnetic measurements. Figures for the CN', SH', and F' derivatives of catalase are given in Table III.

Crystalline horse-radish peroxidase and its derivatives have been examined by Theorell.<sup>42, 43</sup> In this case the total iron (0.127%) is present

<sup>37</sup> *Arkiv Kemi, Min. Geol.*, 1942, **16**, A, No. 1.

<sup>38</sup> *Ibid.*, No. 7.

<sup>39</sup> L. Michaelis and S. Granick, *J. Gen. Physiol.*, 1941, **25**, 325.

<sup>40</sup> R. Lemberg and J. W. Legge, *Biochem. J.*, 1943, **37**, 117.

<sup>41</sup> *Proc. Roy. Soc.*, 1938, *B*, **124**, 397; *Biochem. J.*, 1945, **39**, 148.

<sup>42</sup> *Enzymologia*, 1942, **10**, 250. <sup>43</sup> *Arkiv Kemi, Min. Geol.*, 1942, **16**, A, No. 3.

TABLE III.

	$10^6 \chi_m$	Temp.	$\mu_B$	U.e.
Hæmoglobin (ox blood) .....	12,290	24°	5.43	4
O <sub>2</sub> , CO, NO, CN, EtNC derivatives .....	0		0	0
Methæmoglobin, acid .....	14,040	"	5.80	5
" alkaline .....	8,340	"	4.47	3
" F' .....	14,610	"	5.92	5
" CN' .....	2,610	"	2.50	1
" SH' .....	2,140	"	2.26	1
" iminazole .....	2,940	25	2.66	1
" NH <sub>3</sub> .....	3,700	"	2.93	1
" N <sub>3</sub> .....	3,360	"	2.84	1
" EtOH .....	12,250	"	5.39	5
Myoglobin .....	12,400	24	5.46	4
Metmyoglobin .....	14,200	"	5.85	5
Hæmin (cryst.) .....	average		5.82	5
Hæmatin (hæmin in NaOH) .....	average		3.52	3?
" + sucrose .....	13,080	20	5.56	5
Hæm (hæmatin + Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> ) .....	9,310	19	4.69	4
Parahæmatin (hæmatin + pyridine) .....	1,660	18	1.97	1
Hæmochromogens (pyridine, dicyanide, globin, nicotine) .....	0		0	0
Bromohæmin (solid) .....	14,585	14	5.81	5
Acetoxylæmin (solid) .....	14,618	12	5.80	5
Formoxylæmin (solid) .....	14,941	"	5.86	5
Azahæmin (solid) .....	14,320	14	5.76	5
Hæmin dimethyl ester (solid) .....	14,376	13	5.75	5
Hæmatin (solid) .....	14,569	"	5.79	5
Hæmatin $\frac{1}{2}$ anhydride (solid) .....	15,053	"	5.89	5
Hæmatin anhydride (solid) .....	14,456	"	5.77	5
Peroxidase (pH 4-9) .....	12,560	20	—	5
" F' .....	14,840	"	—	5
" SH' .....	2,440	"	—	1
" CN' .....	2,970	"	—	1
" H <sub>2</sub> O <sub>2</sub> .....	< 4,800	"	—	1?
Reduced peroxidase .....	11,410	"	—	4
" " CO .....	0	"	—	0
Catalase .....	14,665	"	5.89	5
" CN' .....	6,830	"	4.02	3
" N <sub>3</sub> .....	14,500	"	5.86	5
" F' .....	14,665	"	5.89	5
" SH' .....	7,290	"	—	3?
Azide catalase + H <sub>2</sub> O <sub>2</sub> in N <sub>2</sub> .....	6,600	"	3.95	?
" " " CO .....	4,920	"	3.41	?

as hæmatin. The results (Table III) are similar to those obtained with MetHb except that  $10^6 \chi_m$  for free peroxidase in neutral solution is rather low for 5 u.e. (12,650) and in alkaline solution it drops to 2800. The figures given for H<sub>2</sub>O<sub>2</sub> peroxidase are not significant, as a mixture of derivatives is present; nevertheless, covalent bonding is probable. Theorell records a very labile green hydrogen peroxide derivative which changes rapidly to the red hydrogen peroxide peroxidase I of D. Keilin and T. Mann.<sup>44</sup> The CN', SH', and F' derivatives of peroxidase are strictly comparable with those of MetHb.

(G) *Covalent and ionic bonding.* Although magnetic measurements indicate ionic bonding in some hæmatin derivatives, the iron is held more securely than in iron salts. Hence, all tests for the ion are negative and it cannot be removed electrolytically. Furthermore, it has not been

<sup>44</sup> *Proc. Roy. Soc., 1937, B, 122, 119.*



possible to introduce radioactive iron into Hb by ion exchange.<sup>45</sup> The enclosure of the iron atom within the cyclic porphyrin structure with its high resonance energy is no doubt responsible for its inaccessibility. Slight modifications of the porphyrin such as removal of one :CH (bile pigment) or hydrogenation of some double bonds (porphyrinogen) render the iron more labile.

*Analogies between Magnetic and Optical Properties of Hæmatin Derivatives.*—The relationships between absorption spectra and variation in iron bonding have already been outlined.<sup>43</sup> Since the magnetic and spectroscopic approaches to the study of hæmatin compounds must be regarded as complementary, inasmuch as the same processes may in general be followed by both techniques, these relationships deserve special attention. The available data are collected in Table IV.

TABLE IV.

Group.	Fe valency.	Fe bonding.	Colour and spectrum type.	Examples.
a	3	Ionic	Green-brown. Abs. band in red between 600 and 640 m $\mu$ . Strong band in blue, sometimes faint bands in green.	Hæmin in sucrose-NaOH. Hæmin in pyridino. MetHb and F' cpd. Catalase and F' and N <sub>3</sub> ' cpds. Peroxidase and F' cpd.
b	2	Ionic	Carmine-red-purple. Diffuse band in green.	Hæmoglobin. Myoglobin. Hæm.
c	3	Covalent	Bright red; 2 diffuse bands in green.  Brown-red diffuse band in green.	SH', N <sub>3</sub> ', H <sub>2</sub> O <sub>2</sub> cpds. of MetHb. SH', CN' cpds. of peroxidase. Parahæmatins, e.g., cytochrome-c.
d	2	Covalent	Scarlet to pink; 2 very sharp bands in green.	O <sub>2</sub> , CO, NO cpds. of Hb. Hæmochromogens (cyt.-c). Reduced peroxidase-CO.

*Exceptions:* (1) Alkaline MetHb is intermediate between (a) and (c): 3 u.e., red-brown colour, two bands in the green plus a narrow band at 600 m $\mu$ .

(2) MetHbCN falls into group (c) except that the spectrum resembles (b).

(3) Reduced peroxidase falls into group (b) but has two bands in the green.

(4) CN' and SH' compounds of catalase appear to have 3 u.e. Otherwise they resemble the corresponding MetHb compounds [group (c)].

E. F. H.

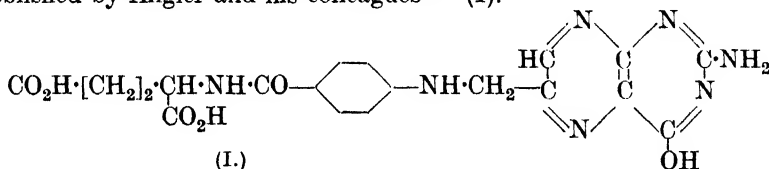
## 5. NUTRITION: ANTI-ANÆMIA FACTORS.

In the last three years a number of substances have been described with properties which justify their inclusion in a single group. Among them are vitamin M, vitamin B<sub>12</sub>, the norite eluate factor, the *L. casei* factor, factor U, folic acid, and the *S. lactis* R. factor. Properties common to most of them are stimulation of bacterial growth and of hæmatopoiesis in mammals including man. It is the latter property which has given these substances prominence in the treatment of human macrocytic anæmia. It is now certain many of these substances share a common structure, varied by the attachment of different chemical groups. The precise relationship among them has still to be established. A certain confusion obvious in papers dealing with

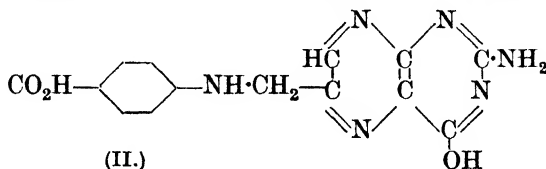
<sup>45</sup> See Selwood, *op. cit.* (ref. 4), p. 171.

these factors lies in the indiscriminate use of names, in attempts to relate those active towards micro-organisms with those active towards animals, and in neglect to state the source of the factors. The confusion is, however, being rapidly dispelled by reports upon the chemical nature of the factors. Meanwhile unambiguous use of names is essential.

No discussion of the animal factors can be truly appreciated without a sketch of those active towards *L. casei* and *S. lactis R.* The name "folic acid" has been used most haphazardly in designating concentrates and substances active towards animals and micro-organisms. In the first instance it was used by Williams<sup>1</sup> to denote a substance isolated in a highly purified form from spinach which stimulated the growth of *S. lactis R.* This substance is also active towards *L. casei*. With these two micro-organisms as test objects, the existence of several active substances has been established. Two factors have been isolated, one from liver and the other from yeast, both equally active towards *L. casei*.<sup>2</sup> But towards *S. lactis R.* the liver factor is twice as active as the yeast one. A third *L. casei* factor has been obtained from a fermentation residue.<sup>3,4</sup> Compared with the liver *L. casei* factor it is 85–90% as active towards *L. casei* and only 6% towards *S. lactis R.* These three factors have been obtained in crystalline form. By degradation and synthesis the structure of the liver *L. casei* factor has been established by Angier and his colleagues<sup>4, 5</sup> (I).



The presence in the molecule of the *p*-aminobenzoyl group is of interest because of its antagonistic effects upon sulphonamides, and that of the 2-amino-6-hydroxypteridine structure because of the many hints that the



pterins have a part in hæmatopoiesis. Angier *et al.* recommend an acceptable nomenclature based upon pteric acid (II), which, if adopted, would simplify

<sup>1</sup> H. K. Mitchell, E. E. Snell, and R. J. Williams, *J. Amer. Chem. Soc.*, 1941, **63**, 2284.

<sup>2</sup> E. L. R. Stokstad, *J. Biol. Chem.*, 1943, **149**, 573.

<sup>3</sup> B. L. Hutchings, E. L. R. Stokstad, N. Bohonos, and N. H. Stobodkin, *Science*, 1944, **99**, 371.

<sup>4</sup> R. B. Angier, J. H. Boothe, B. L. Hutchings, J. H. Mowat, J. Semb, E. L. R. Stokstad, Y. SubbaRow, C. W. Waller, D. B. Cosulich, M. J. Jahrenbach, M. E. Hultquist, E. Kuh, E. H. Northey, D. R. Seeger, J. P. Sickels, and J. M. Smith, *ibid.*, 1945, **102**, 227.

<sup>5</sup> *Ibid.*, 1946, **103**, 667.

the existing terminology of these new anti-anæmic factors. The liver *L. casei* factor would be named pteroylglutamic acid. The fermentation *L. casei* factor contains <sup>5</sup> two extra glutamic acid residues; probably it is pteroyldiglutamylglutamic acid. Another product was obtained by Angier *et al.* by the same method of synthesis used for the *L. casei* factor, by the condensation of *p*-aminobenzoic acid with *N*-(2-amino-6-hydroxy-8-pteridyl)methylpyridinium iodide. Unlike the other two substances, it is active towards *S. lactis R.* but not towards *L. casei* or the chick. It will be interesting if this product turns out to be identical with pteric acid and the *S. lactis R.* factor of Keresztesy and his co-workers.<sup>6</sup>

Synthetic *L. casei* factor is active in preventing anæmia in chicks.<sup>5</sup> It is highly probable that other crystalline products active in the same respect are identical with or closely related to pteroylglutamic acid. Pfiffner and his co-workers <sup>7, 8, 9</sup> have isolated two compounds in crystalline form; one, an orange coloured acid, named vitamin B<sub>12</sub>, and a second from yeast, which has been named vitamin B<sub>12</sub> conjugate. Both have anti-anæmic activity. Vitamin B<sub>12</sub> conjugate has a molecular weight of *ca.* 1400, roughly 2—3 times that of vitamin B<sub>12</sub>, and a spectral absorption very similar to vitamin B<sub>12</sub>. From hydrolysis experiments and electrophoretic behaviour, the conjugate has been shown to contain 7 glutamic acid residues,<sup>9</sup> a fact which relates it to the fermentation *L. casei* factor. It is, however, almost inactive microbiologically, which differentiates it from the *L. casei* factor and vitamin B<sub>12</sub>. But on incubation with an enzyme, named vitamin B<sub>12</sub> conjugase, the conjugate yields vitamin B<sub>12</sub> which is microbiologically active. Following the recommendations of Angier *et al.*, Pfiffner and his co-workers have renamed the conjugate pteroylhexaglutamylglutamic acid.

The isolation of conjugates of vitamin B<sub>12</sub> and of the liver *L. casei* factor provides an explanation of the different effects upon micro-organisms of concentrates of the factors and of partly purified substances. Illustrative of this point is the difference in activity of the liver *L. casei* factor, the fermentation *L. casei* factor, and pteroylhexaglutamylglutamic acid towards *L. casei*. The enhanced microbiological activity of concentrates of the factor after enzymatic digestion obviously results from the conversion of conjugates into forms utilisable by micro-organisms. This conversion can be effected by the enzyme vitamin B<sub>12</sub> conjugase,<sup>10</sup> widely distributed in animal tissues. Some mention of what is known of this enzyme is worth while, since it may play a part in the utilisation of conjugates by animals and micro-organisms.<sup>11</sup> The method of testing conjugase activity consists in incubating

<sup>6</sup> B. C. Keresztesy, E. L. Rickes, and J. L. Stokes, *Science*, 1943, **97**, 465.

<sup>7</sup> J. J. Pfiffner, S. B. Binkley, E. S. Bloom, R. A. Brown, O. D. Bird, A. D. Emmett, A. G. Hogan and B. L. O'Dell, *ibid.*, p. 404.

<sup>8</sup> *Ibid.*, 1945, **102**, 228.

<sup>9</sup> J. J. Pfiffner, D. G. Calkins, E. S. Bloom, and B. L. O'Dell, *J. Amer. Chem. Soc.*, 1946, **68**, 1392.

<sup>10</sup> O. D. Bird, S. B. Binkley, E. S. Bloom, A. D. Emmett, and J. J. Pfiffner, *J. Biol. Chem.*, 1945, **157**, 413.

<sup>11</sup> O. D. Bird and M. Robbins, *ibid.*, 1946, **163**, 661.

extracts of tissues with concentrates or preparations of vitamin B<sub>6</sub> conjugate, and the estimation of the amount of *S. lactis R.* factor in the digest.<sup>12</sup> Conjugase activity is shown by kidney, liver, pancreas, and intestine of animals and birds, by almonds, by potatoes, and, to a slight extent, by moulds.<sup>10, 13</sup> Conjugase activity is not shown by phosphatase, nucleosidase, or  $\beta$ -glucosidase.<sup>10, 14</sup> Chicken pancreas has a high conjugase content, whilst hog's kidneys are a good starting material for enzyme preparations.<sup>10</sup> From kinetic studies and determinations of optimum pH values, the activity of tissues may be due to more than one conjugase,<sup>15, 16</sup> which may be found to differ in their mode of action. But from comparative studies with crystalline vitamin B<sub>6</sub> conjugate it would seem that the substrates attacked by the conjugases are structurally akin to the conjugates and do yield vitamin B<sub>6</sub>. More precise information upon these enzymes will be welcome, since their absence or inhibition in the gut may be a factor in human macrocytic anæmia in which defects in intestinal absorption are a feature.

Several factors are effective in preventing anæmia in chicks. They are the *L. casei* factor (from liver and the synthetic pteroylglutamic acid), the fermentation *L. casei* factor<sup>17</sup> (pteroyldiglutamylglutamic acid), vitamin B<sub>6</sub>, and the vitamin B<sub>6</sub> conjugate (pteroylhexaglutamylglutamic acid). In biological and chemical properties vitamin B<sub>6</sub> is very similar to *L. casei* factor. The fact that Pfiffner *et al.* designate vitamin B<sub>6</sub> conjugate as pteroylhexaglutamylglutamic acid, taken with their observation of the presence of glutamic acid in vitamin B<sub>6</sub>, suggests that they believe vitamin B<sub>6</sub> to be pteroylglutamic acid. Tested microbiologically, vitamin B<sub>6</sub>, pteroylglutamic acid, and folic acid have the same activity towards *L. casei*<sup>18</sup> and *S. lactis R.*, from which it has been concluded that they are one and the same substance. It would seem that the active component is pteroylglutamic acid into which the conjugated forms are converted, possibly by the action of the conjugases within the intestine.

C. F. Campbell, M. M. McCabe, R. A. Brown, and A. D. Emmett<sup>19</sup> have described in detail the hæmatological changes which occur in chicks as the results of vitamin B<sub>6</sub> deficiency. After three weeks on the purified diet the chicks showed very poor feathering. At about the same time there was a definite anæmia characterised by macrocytosis and normoblasts, pronormoblasts and myeloblasts in the blood, a leukopenia, and a thrombopenia. These severe changes in the blood cells were prevented by diets containing 100  $\mu$ g. of crystalline vitamin B<sub>6</sub>/100 g. of diet. Not all workers agree that vitamin B<sub>6</sub> alone can prevent anæmia in the chick. M. L. Scott, L. C. Norris,

<sup>12</sup> V. Mims, J. R. Totter, and P. L. Day, *J. Biol. Chem.*, 1944, **155**, 401.

<sup>13</sup> M. Laskowski, V. Mims, and P. L. Day, *ibid.*, 1945, **157**, 731.

<sup>14</sup> V. Mims and M. Laskowski, *ibid.*, **159**, 493.

<sup>15</sup> J. G. Memon and J. R. Totter, *ibid.*, p. 301.

<sup>16</sup> O. D. Bird, M. Robbins, J. M. Vandenbelt, and J. J. Pfiffner, *ibid.*, 1946, **163**, 649.

<sup>17</sup> B. L. Hutchings, J. J. Oleson, and E. L. R. Stokstad, *ibid.*, p. 447.

<sup>18</sup> B. C. Johnson, *ibid.*, p. 255.

<sup>19</sup> *Amer. J. Physiol.*, 1945, **144**, 348.

G. F. Heuser, and W. F. Bruce<sup>20</sup> consider that either  $\alpha$ - or  $\beta$ -pyracin is also necessary. The adjuvant effect of pyracin has not been observed by others.<sup>17</sup> The discrepancy is not resolved by the suggestion, based on the *in vitro* conversion of crystalline *L. casei* factor into *S. lactis R.* factor by liver, that pyracin is conjugated with *L. casei* factor or part of enzyme responsible for the conversion.<sup>21</sup>

There are a number of problems of chick nutrition still to be solved. One at least appears to have been settled, and that is the nature of the factor concerned with the feathering of chicks. Elvehjem and his co-workers<sup>22</sup> reported the presence of two factors in the norite eluate concentrations; vitamin B<sub>10</sub> responsible for good feathering and vitamin B<sub>11</sub> essential for growth. Crystalline vitamin B<sub>6</sub> has been shown to prevent poor feathering.<sup>23</sup> Oleson and his co-workers have shown that pteroylglutamic acid added to synthetic diet ensures good feathering and that other factors such as ascorbic acid and *p*-aminobenzoic acid are unnecessary.<sup>24</sup> It seems fair to conclude that vitamin B<sub>10</sub> is very similar to pteroylglutamic acid. The relation of the antianæmic factors to feathering is not without interest when it is remembered that poor hair and nail growth is a feature of human macrocytic anæmia.

It is impossible to discuss at length the extensive work upon the relation of the antianæmic factors to the good health of the rat. One or two phases may be selected to illustrate other properties of the pteridines. Our understanding of agranulocytosis and of acute granulocytopenia associated with the administration of drugs is poor. In rats a profound disturbance of growth and of blood formation characterized by agranulocytosis and hypocellularity of blood marrow develops from the inclusion in the diet of sulphaguanidine and sulphasuxidine.<sup>25,26</sup> These effects can be remedied by the feeding of liver extracts rich in "folic acid" or by crystalline "folic acid".<sup>26,27,28</sup> In a small number of rats upon purified diets agranulocytosis develops without the administration of sulphonamides.<sup>29</sup> This also responds to *L. casei* factor. Daft and his co-workers<sup>30</sup> observed more severe blood disorders in rats fed a purified diet low in pantothenic acid. Pantothenic acid deficiency did not manifest itself uniformly in a particular group of rats. In some rats

<sup>20</sup> *Amer. J. Physiol.*, **153**, 291.

<sup>21</sup> L. J. Daniel, M. L. Scott, L. C. Norris, and G. F. Heuser, *ibid.*, **160**, 265.

<sup>22</sup> G. M. Briggs, T. D. Luckey, C. A. Elvehjem, and E. B. Ward, *ibid.*, 1943, **148**, 163; 1944, **153**, 423.

<sup>23</sup> C. J. Campbell, R. A. Brown, and A. D. Emmett, *ibid.*, **152**, 483.

<sup>24</sup> J. J. Oleson, B. L. Hutchings, and N. A. Sloane, *ibid.*, 1946, **165**, 371.

<sup>25</sup> A. D. Welch, P. A. Wattis, and A. R. Latven, *J. Pharm. Exp. Ther.*, 1942, **75**, 231.

<sup>26</sup> S. S. Spicer, F. S. Daft, W. H. Sebrell, and L. L. Ashburn, *Publ. Health Reps., Wash.*, 1942, **57**, 7559.

<sup>27</sup> A. Kornberg, F. S. Daft, and W. H. Sebrell, *Science*, 1943, **98**, 20.

<sup>28</sup> F. S. Daft and W. H. Sebrell, *Publ. Health Reps., Wash.*, 1943, **58**, 1542.

<sup>29</sup> A. Kornberg, F. S. Daft, and W. H. Sebrell, *Proc. Soc. Exp. Biol. Med.*, 1945, **58**, 46.

<sup>30</sup> F. S. Daft, A. Kornberg, L. L. Ashburn, and W. H. Sebrell, *Publ. Health Reps., Wash.*, 1945, **60**, 1201.

granulocytopenia occurred together with anæmia; in others, anæmia was the presenting symptom; in a few, granulocytopenia, and in some, no blood dyscrasia. The most severe anæmia was seen in the granulocytopenic rats and was accompanied by hypoplasia of the bone marrow. In the anæmic animals, hypoplasia of the marrow was less frequent and less severe. None of these blood disorders develops in the control animals which receive pantothenic acid. Despite its prophylactic effectiveness, pantothenic acid produced a slow cure of the anæmia and had a slight effect upon the granulocytopenia. On the view that pantothenic acid deficiency had produced a deficiency of another factor, the fermentation *L. casei* (or liver *L. casei*) factor was administered together with pantothenic acid. This treatment proved far more effective in curing the blood dyscrasias than that of either factor alone. Results similar to, although not identical with, those of Daft and his co-workers were obtained by Carter and his co-workers.<sup>31</sup> In their rats a deficiency of pantothenic acid led to a hypochromic anæmia and a reduction in polymorphonuclear leucocytes. The bone marrow showed hyperplasia and evidence of failure of maturation of both erythropoietic and leucopoietic cells. Initiated at an early stage of the disease, pantothenic acid therapy produced a restoration of a normal blood picture. Furthermore, the control rats receiving pantothenic acid developed an anæmia after a prolonged period which might be attributed to the lack of anti-anæmic factor of the pteridine type. The explanation of these findings is not easy. It may be that a deficiency of pantothenic acid conditions a deficiency of "folic acid." It is to be noted that L. D. Wright and A. D. Welch<sup>32</sup> have considered that both biotin and "folic acid" may perhaps be essential for the storage or utilization of pantothenic acid.

For many years it has been recognised that inadequate diet produces a profound and often fatal disorder of the blood in the monkey.<sup>33, 34, 35</sup> The blood picture of the animals is one of anæmia and leucopenia. The deficient animals usually suffer from ulcerated gums and from diarrhœa and they become easily susceptible to spontaneous infection. Untreated, the condition progresses to a fatal end. Yeast and yeast products and liver are curative and often elicit a reticulocyte response. Since none of the well-known members of the vitamin B complex or other vitamins<sup>36, 37</sup> affects the condition, it has been attributed to lack of a factor known as vitamin M. Recently, preparations of "folic acid" have proved effective in treatment.<sup>38</sup> Some success had been obtained with xanthopterin, which produced a sub-

<sup>31</sup> C. W. Carter, R. G. Macfarlane, J. R. P. O'Brien, and A. H. T. Robb-Smith, *Biochem. J.*, 1945, **39**, 339.

<sup>32</sup> *J. Nutrition*, 1944, **27**, 55.

<sup>33</sup> L. Wills and H. S. Bilimoria, *Indian J. Med. Res.*, 1932, **20**, 291.

<sup>34</sup> L. Wills and A. Stewart, *Brit. J. Exp. Path.*, 1935, **16**, 444.

<sup>35</sup> P. L. Day, W. C. Langston, and C. F. Shukers, *J. Nutrition*, 1935, **9**, 637.

<sup>36</sup> W. C. Langston, W. J. Darby, C. F. Shukers, and P. L. Day, *J. Exp. Med.*, 1938, **68**, 923.

<sup>37</sup> S. Saslaw, H. F. Wilson, C. A. Doan, and J. L. Schwab, *Science*, 1943, **97**, 514.

<sup>38</sup> H. A. Waisman and C. A. Elvhjem, *J. Nutrition*, 1943, **28**, 361.

normal reticulocyte response and a return of blood cells to normal.<sup>39</sup> The effect of the pterin did not persist unless given together with liver powder. The most effective treatment has been with highly purified preparations of *L. casei* factor or with crystalline *L. casei* factor.<sup>40,41</sup> Intramuscular injections of crystalline *L. casei* factor produces a reticulocyte response as high as 47% within 4—7 days and restoration of the number of red and white cells to normal and a definite clinical improvement. The remarkable success of *L. casei* factor in the treatment of nutritional anæmia of the monkey and similar conditions in other species indicates rather strongly that vitamin M falls in the class of the pteroylglutamic acids. This view is supported by the fact that, although the folic acid content (as measured by the growth of *S. lactis R.*) of substances with vitamin M activity is low, it parallels the vitamin M potency of substances after they have been incubated with rat's liver.<sup>42</sup>

The therapeutic success of the *L. casei* factors in the treatment of anæmia and leucopenia in animals justified their clinical trial in cases of macrocytic anæmia. There were also other reasons. In some respects vitamin deficiency in the monkey is analogous to sprue in man. Furthermore it has long been suspected that an unknown factor of that group of miscellaneous substances, the vitamin B complex, has a rôle in those macrocytic anæmias the origin of which is nutritional deficiency. Among these anæmias may be included those called refractory because of their unresponsiveness to the usual therapeutic measures such as purified liver extracts and iron. Typical examples are refractory anæmias of pregnancy and malnutrition. Related to these on hæmatological grounds are the anæmias of sprue and Addisonian pernicious anæmia. The existence of anti-anæmic factor was indicated by the curative action of crude yeast and liver preparations upon macrocytic anæmia of pregnancy and tropical macrocytic anæmia<sup>43,44</sup> and by the beneficial effect of dried yeast upon pernicious anæmia.<sup>45</sup> In the last five years the investigation of anæmia of pregnancy has been pursued most diligently. But despite its similarity to pernicious anæmia, from which it is most readily distinguished by free hydrochloric acid in the gastric juice, pregnancy anæmia responds only to the most vigorous therapeutic treatment,<sup>46</sup> usually with liver in an unpurified form.<sup>47,48</sup> It would seem that

<sup>39</sup> J. R. Totter, C. F. Shukers, J. Kolson, V. Mims, and P. L. Day, *J. Biol. Chem.*, 1944, **152**, 147.

<sup>40</sup> P. L. Day, V. Mims, J. R. Totter, E. L. R. Stokstad, B. L. Hutchings, and N. H. Sloane, *ibid.*, 1945, **157**, 423.

<sup>41</sup> P. L. Day, V. Mims and J. R. Totter, *ibid.*, **161**, 45.

<sup>42</sup> J. R. Totter, V. Mims, and P. L. Day, *Science*, 1944, **100**, 223.

<sup>43</sup> L. Wills, *Brit. Med. J.*, 1931, i, 1059.

<sup>44</sup> L. Wills and B. D. F. Evans, *Lancet*, 1938, ii, 416.

<sup>45</sup> M. Wintrobe, *Amer. J. Med. Sci.*, 1939, **197**, 286.

<sup>46</sup> L. S. P. Davidson, L. J. Davis, and J. Innes, *Brit. Med. J.*, 1942, ii, 31.

<sup>47</sup> H. W. Fullerton, *ibid.*, 1943, i, 158.

<sup>48</sup> J. Watson and W. B. Castle, *Proc. Soc. Exp. Biol. Med.*, 1945, **58**, 84; *Amer. J. Med. Sci.*, 1946, **211**, 513.

pregnancy anæmia and other macrocytic anæmias associated with malnutrition are the consequence of the lack of a substance which is not the anti-pernicious anæmia factor.<sup>48</sup> This factor may be related to pteroylglutamic acid or one of its several forms. For, in the last two years, a number of reports have appeared upon the beneficial effect of the synthetic *L. casei* factor upon macrocytic anæmias of differing ætiology. The claims made in some of the first reports would have been more convincing had they been accompanied by a statement of the criteria of diagnosis and data upon the changes in the bone marrow. Moreover, no reports have been made of follow-ups of treated cases to allow a judgment of how lasting is the effect of *L. casei* factor. Nevertheless it would seem that, in pteroylglutamic acid in one form or another, we have a therapeutic agent of value.

Evidence for the hæmopoietic activity of synthetic *L. casei* factor is accumulating. In 1945 Spies and his co-workers<sup>49</sup> reported a hæmatologic response in nine unspecified cases of macrocytic anæmia following the administration of synthetic *L. casei* factor. During treatment the patients were given a diet free from meat and meat products to reduce their intake of the extrinsic factor. Given intravenously or orally, the compound produced a reticulocytosis and a rise in hæmoglobin and red cells. A second report by Spies and his co-workers<sup>50</sup> describes the effect of the synthetic material upon fourteen cases of macrocytic anæmia; nutritional macrocytic anæmia (6), Addisonian pernicious anæmia (5), and indeterminate (3). In these and in others<sup>51</sup> a full restoration of hæmoglobin and red cells to normal values is not always observed. Moore and his co-workers<sup>52</sup> also describe remissions in two cases of pernicious anæmia and one case of anæmia of pregnancy following the administration of synthetic *L. casei*. In all three cases there was a reticulocyte response of 40—50%, and a rise in hæmoglobin and red cells with doses of 20—100 mg. of synthetic *L. casei* factor given daily for 10—15 days. It is to be noted that a total dose of 1 g. of synthetic compound was insufficient to produce a complete remission in one of the cases of pernicious anæmia. On the other hand a dose of 2 mg. of "folic acid" given daily for 20 days is stated to produce complete remission in a case of Addisonian pernicious anæmia.<sup>53</sup> There can be little doubt that in the macrocytic anæmia the synthetic *L. casei* factor has a hæmopoietic action and its use in the treatment of macrocytic anæmia of pregnancy and malnutrition may be valuable. In pernicious anæmia it has not produced complete remission in the amounts in which it occurs in therapeutic doses of liver extract,<sup>54</sup> or a hæmopoietic response in amounts of 0.7 mg.—a dose in which highly purified

<sup>48</sup> T. A. Spies, C. F. Vilter, M. B. Koch, and M. H. Caldwell, *South Med. J.*, 1945, 38, 707.

<sup>49</sup> C. F. Vilter, T. D. Spies, and M. D. Koch, *ibid.*, p. 781.

<sup>51</sup> T. D. Spies, *Lancet*, 1946, i, 225.

<sup>52</sup> C. V. Moore, O. S. Bierbaum, A. D. Welch, and L. D. Wright, *J. Lab. Clin. Med.*, 1945, 30, 1056.

<sup>53</sup> C. A. Doan, H. E. Wilson, and C. S. Wright, *Ohio State Med. J.*, 1946, 42, 139.

<sup>54</sup> G. W. Clark, *Amer. J. Med. Sci.*, 1945, 209, 520.



liver extracts are active.<sup>55</sup> Probably there are two or more factors, one associated with the defect in pernicious anæmia and the others with the defects in the nutritional macrocytic anæmias. The similar biological effects of the anti-pernicious factor and the *L. casei* factor may possibly be due to their having in common a group such as that of the pterins.<sup>56</sup>

In tropical and non-tropical sprue, synthetic *L. casei* factor has a beneficial effect.<sup>57, 58, 59, 60, 61</sup> Most of the cases which have been treated fulfil the diagnostic criteria for sprue<sup>62</sup> in that they showed a macrocytic anæmia, leucopenia, glossitis, diarrhoea with increased fat in stools, loss of weight, pigmentation of the skin, etc. The presence of free hydrochloric acid in the gastric juice differentiated them from pernicious anæmia. The typical response to pteroylglutamic acid is as follows. The intramuscular administration of 15 mg. of synthetic *L. casei* factor daily is followed within a few days by a reticulocyte response and rise in hæmoglobin, red and white cells, and platelets. This hæmatologic response is accompanied by a definite improvement in the clinical condition. Glossitis disappears, diarrhoea subsides, and appetite improves. Studies of the bone marrow<sup>57, 58</sup> show that the primitive red cells present before treatment disappear and the white cell series return to normal. In most of the cases the response to treatment is rapid and even dramatic, and, in some, most effective in that the patients remain in excellent health.<sup>61</sup> Most probably the beneficial effect of liver extract upon sprue can be ascribed to the presence of a substance allied to or identical with pteroylglutamic acid. The close similarity of vitamin M deficiency in the monkey to sprue may permit this disease to be attacked more vigorously from the experimental side.

#### *Pterins and the Macrocytic Anæmia Factors.*

The close link now established between the *L. casei* factors and the hæmatopoietic system gives a new significance to the pterins, the pigments of the wings of insects, for it is now certain that the *L. casei* factors contain within their molecules the pteridine group. It may be said that without information of the biological effects of the pterins and of their chemical structure the elucidation of the nature of the *L. casei* factors would not have been so speedily achieved. Of these pigments, which, as early as 1889<sup>63</sup>

<sup>55</sup> Y. SubbaRow, A. H. Hastings, M. Elkins, "Vitamins and Hormones", Vol. 3, Academic Press Inc., New York, 1945, 237.

<sup>56</sup> W. Jacobson and D. M. Simpson, *Biochem. J.*, 1946, **40**, 3.

<sup>57</sup> W. J. Darby and E. Jones, *Proc. Soc. Exp. Biol. Med.*, 1945, **60**, 259.

<sup>58</sup> W. J. Darby, E. Jones, and H. C. Johnson, *Science*, 1946, **103**, 108.

<sup>59</sup> T. D. Spies, F. Milanes, J. A. Menendez, and V. Mennich, *J. Lab. Clin. Med.* 1945, **30**, 1056.

<sup>60</sup> T. D. Spies, V. Minnich, M. Koch, G. G. Lopez, and J. H. Menendez, *South Med. J.*, 1946, **39**, 30.

<sup>61</sup> G. G. Lopez, T. D. Spies, J. A. Menendez, and R. L. Toca, *J. Amer. Med. Assoc.*, 1946, **132**, 906.

<sup>62</sup> F. M. Hanes, *Amer. J. Med. Sci.*, 1942, **204**, 436.

<sup>63</sup> F. G. Hopkins, *Proc.*, 1889, **5**, 117.

and as late as 1941,<sup>64</sup> were investigated by Hopkins, two, xanthopterin and leucopterin, have been synthesised. From several lines of evidence they may be involved in the processes of hæmatopoiesis. R. Tschesche and H. J. Wolf<sup>65</sup> claim that the injection of 10 µg. of xanthopterin cures the anæmia of rats produced by feeding goat's milk. The anæmia of trout fed on deficient diets also responds to natural and synthetic xanthopterin.<sup>66</sup> The same pigment restores but does not maintain a normal picture in vitamin M-deficient monkeys<sup>39</sup> and is also beneficial to rats which, having ingested succinylsulphathiazole, have developed leucopenia.<sup>67</sup> These effects may be mediated by xanthopterin *per se*. On the other hand, the trout, the rat, and the monkey may be capable of synthesising the active hæmatopoietic factor from the pterin.

Xanthopterin is present in mammalian tissues, where it may exercise an enzymatic rôle,<sup>68</sup> perhaps similar to the flavins. It is present in liver<sup>69</sup> and liver extracts<sup>70</sup> and is excreted in the urine of man.<sup>71</sup> More interesting is the observation of Jacobson<sup>72</sup> that the argentaffin cells of the mucosa of the digestive tract contain pterins. These specially differentiated cells lie in the cardiac and pyloric areas of the stomach and in the intestine, particularly the duodenum. There is a similarity in the distribution of these cells and those from which the anti-pernicious anæmia factor can be obtained. In autopsy specimens obtained from twelve cases of pernicious anæmia the argentaffin cells were absent or reduced in numbers.<sup>72</sup> More recently W. Jacobson and D. M. Simpson<sup>73</sup> have compared the fluorescence spectra of the cytoplasmic granules of argentaffin cells with those of xanthopterin and leucopterin. They found that extracts of cells had a spectrum almost identical with that of xanthopterin. On the other hand the fluorescence spectra of eighteen commercial and experimental liver extracts, all active against pernicious anæmia, indicated the presence of leucopterin or a mixture of leucopterin and some xanthopterin.<sup>74</sup> Furthermore the hæmopoietic activity of the extracts, assessed by their action upon cases of pernicious anæmia or upon splenectomised rabbits<sup>75</sup> and the intensity of their fluorescence, run parallel. This would suggest that the hæmatopoietic activity and fluorescence arise from the same substance—the anti-pernicious anæmia factor. Jacobson and Simpson<sup>74</sup> consider that this factor contains pterin bound to some other substance. Without further data it is premature to speculate upon reconciliation of these findings with those upon factors

<sup>64</sup> F. G. Hopkins, *Proc. Roy. Soc.*, 1942, B, **130**, 359.

<sup>65</sup> *Z. physiol. Chem.*, 1937, **248**, 34.

<sup>66</sup> R. W. Simmons and E. R. Norris, *J. Biol. Chem.*, 1941, **140**, 679.

<sup>67</sup> J. R. Totter and P. L. Day, *ibid.*, 1943, **147**, 257.

<sup>68</sup> W. Koschura, *Z. physiol. Chem.*, 1937, **250**, 161.

<sup>69</sup> *Ibid.*, 1936, **240**, 127.

<sup>70</sup> B. M. Jacobson and Y. SubbaRow, *J. Clin. Invest.*, 1937, **16**, 373.

<sup>71</sup> W. Koschura, *Z. physiol. Chem.*, 1943, **277**, 159.

<sup>72</sup> W. Jacobson, *J. Path. Bact.*, 1939, **49**, 1.

<sup>73</sup> *Biochem. J.*, 1946, **40**, 3.

<sup>74</sup> *Ibid.*, p. 9.

<sup>75</sup> *Idem*, *J. Path. Bact.*, 1945, **57**, 423.

known to contain the pteridine group, especially as W. B. Emery and L. F. J. Parker <sup>76</sup> could find no specific ultra-violet absorption characteristics in a highly purified preparation of the anti-pernicious anæmia factor made from liver.

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<sup>76</sup> *Biochem. J.*, 1946, **40**, Proc. iv.

## ANALYTICAL CHEMISTRY.

### 1. INTRODUCTION.

THE various branches of analytical chemistry are seen to be inter-related when it is admitted that any property, physical or chemical, possessed by a substance may be used to identify that substance, and to determine the proportion of that substance in a mixture. These topics are referred to in over 2500 abstracts published in Section C of *British Abstracts* in 1946, and it is manifestly impossible to report adequately in a few pages on the important work which many of these and other publications represent.

A coherent impression of the progress made in a branch of analytical chemistry may be presented either by considering means of identifying and determining members of groups of substances (such as the constituents of coal-tar distillates) or by describing analytical techniques and illustrating their applications by examples drawn from the various fields of chemistry in which they have been applied: such techniques are mass spectrometry and infra-red absorption spectrophotometry, which are referred to extensively for the first time under Analytical Chemistry. Trends are indicated in the application of spectrography, colorimetry, turbidity measurements, polarography, and X-ray diffraction to the determination of small quantities, with emphasis on the importance of characterisation of physical state in addition to determinations of composition.

J. R. N.

### 2. CONSTITUENTS OF COAL-TAR DISTILLATES.

Until comparatively recent years, the analysis of the various fractions and products distilled from coal-tar has been, like the analysis of petroleum products, largely in terms of broad classes of constituents, for example, the total response to bromination or sulphonation as a conventional measure of the content of olefins or aromatics. Modern technological methods, however, leading to the isolation on a practical scale of a greatly extended number of individual compounds of actual or potential commercial use,<sup>1, 2</sup> make it possible to think of the coal-tar distillates much more in terms of pure constituents than was formerly the case. There is thus a corresponding call for analytical methods for the determination of particular compounds, *e.g.*, the individual cresols in mixed isomers, if these are to be used for condensation to plastic resins, or indene in distillation cuts intended for the same purpose; indole, in the highly purified state required for perfumery; or toluene for nitration. In other cases analytical methods are required for the determination of undesirable constituents in products of industrial use and importance, *e.g.*, thiophen in benzene, aniline in dye intermediates, or *o*-hydroxy-

<sup>1</sup> O. Kruber, *Angew. Chem.*, 1940, 53, 69; *Ber.*, 1941, 74, 1688.

<sup>2</sup> E. A. Coulson and J. I. Jones, *Ind. Chem.*, 1946, 579.

diphenyl in phenol (as a distinction of the synthetic from the natural material). In the present report an attempt has therefore been made to survey methods published over a space of about ten years, which appear applicable to the determination of individual compounds which occur in the coal-tar distillates.

These compounds are, of course, frequently encountered in mixtures of considerable complexity, often with homologues or other compounds of closely similar properties. It may therefore be understood as a general rule that such specific methods as are available will be applied only after the completest practicable preliminary separation by solvent extraction, fractional distillation, etc. Improved analytical techniques in fractionation have been discussed fairly recently by J. W. J. Fay,<sup>3</sup> and a further contribution by W. J. Gooderham<sup>4</sup> may also be noted. Much similar work has been done in the nearly-related field of petroleum hydrocarbons.<sup>5</sup> Adsorption also, usually on silica gel, has been applied to the separation of hydrocarbons.<sup>6, 7, 8</sup> Filtration of light hydrocarbons through a column of silica gel retains the aromatic constituents but allows the passage of paraffins, olefins, and naphthenes, which are preferably washed through with a light paraffin such as pentane. The aromatics are desorbed by means of methyl alcohol, and, when this has been removed by washing with water, are recovered quantitatively and can be further separated by fractionation. Active carbon, oxides of magnesium and aluminium, and "Filtrol" have been used as alternatives to silica.

A chromatographic concentration of anthracene in anthracene oils is advocated by F. R. Cropper and N. Strafford,<sup>9</sup> adsorption being effected on active alumina, with a 1 : 4 mixture of chlorobenzene and light petroleum as solvent and developing medium.

Among the hydrocarbons, even after preliminary separation by such methods as the foregoing, few compounds exhibit sufficiently characteristic reactivity to allow of specific analysis by chemical means. Considerable work has, however, been directed to the elaboration of the colorimetric reactions of nitrated aromatic hydrocarbons with a ketone in alkaline conditions.<sup>10</sup> B. H. Dolin<sup>11</sup> reported that, when the nitrated hydrocarbons are treated with butanone, colours are given by benzene, toluene, and the xylenes, but only that due to benzene persists on acidification with acetic acid. H. D. Baernstein<sup>12</sup> eliminates toluene from the nitrated mixture by oxidation to dinitrobenzoic acid, which does not react with butanone; hence

<sup>3</sup> *Ann. Reports*, 1943, **40**, 224.

<sup>4</sup> *J. Soc. Chem. Ind.*, 1944, **63**, 65r.

<sup>5</sup> *E.g.*, H. J. Hepp and D. E. Smith, *Ind. Eng. Chem. (Anal.)*, 1945, **17**, 579.

<sup>6</sup> B. J. Mair and A. F. Forziati, *J. Res. Nat. Bur. Stand.*, 1944, **32**, 151, 165.

<sup>7</sup> N. C. Turner, *Oil and Gas J.*, 1943, **41**, 48.

<sup>8</sup> P. Harteck and K. A. Sulz, *Chemie*, 1943, **56**, 120. Certain hydrocarbons, *e.g.*, *n*-heptane and toluene, have also been separated on zeolites by the "molecular sieve" technique of R. M. Barrer (*J. Soc. Chem. Ind.*, 1945, **64**, 131).

<sup>9</sup> *Ibid.*, 1944, **63**, 268r.

<sup>10</sup> J. Peltzer, *Chem.-Ztg.*, 1933, **57**, 162.

<sup>11</sup> *Ind. Eng. Chem. (Anal.)*, 1943, **15**, 242; *cf. Chem. Abs.*, 1946, **40**, 6023.

<sup>12</sup> *Ind. Eng. Chem. (Anal.)*, 1943, **15**, 251.

a combination of this procedure with that of Dolin makes it possible to estimate benzene, toluene, and xylenes in the presence of each other. M. S. Bykhovskaya<sup>13</sup> estimates benzene and toluene vapours when present together in air, by nitration in conditions leading to the formation of dinitrobenzene and trinitrotoluene. The latter is measured directly by its colour with alcoholic potash. For the benzene estimation, the trinitrotoluene is eliminated by hydrolysis to phenolic compounds; these are separated, by partition between aqueous alkali and an organic solvent, from the dinitrobenzene, which is then estimated by the colour developed with potash and acetone. R. P. Marquardt and E. N. Luce<sup>14</sup> apply the reaction to determine *o*-xylene in hydrocarbon liquors containing monoalkyl- and alkenyl-benzenes such as styrene. The olefinic compounds are eliminated by steam-distillation following mercuration, which renders them non-volatile. The distilled alkyl-benzenes are nitrated and treated with potash and acetone, whereupon the monoalkylbenzenes give a blue colour fading to red, and the xylenes a stable green. The fading of the monoalkylbenzene colour is expedited by addition of ethanolamine, after which the intensity of the green element is measured photo-electrically.

The standard gravimetric nitration method, due to H. P. Reichel, for determination of *m*-xylene, has been extended<sup>15</sup> by the same author so as to permit of the determination of *p*-xylene also. Under the specified conditions (mixed nitric and sulphuric acids in glacial acetic acid) *m*-xylene is nitrated quantitatively to the trinitro-derivative, which is recovered by crystallisation from acetone, with an allowance for its small solubility in the cold solvent. *p*-Xylene is nitrated only in part to the trinitro-derivative, but the yield of this is a constant fraction (71.4%) of the theoretical. It may therefore be used as a measure of the *p*-xylene present, being recovered by evaporation from the acetone liquors, and recrystallised from alcohol, with allowance as before for its small solubility. The nitration products of *o*-xylene and ethylbenzene, being soluble in alcohol, do not interfere. By an analogous procedure, Reichel determines mesitylene, as its acetone-insoluble trinitro-derivative, in the coal-tar spirit fraction of boiling range 160—180°.

Chemical determinations of naphthalene are for practical purposes limited to those based on formation of the picrate, after elimination of other picrate-forming substances. The earlier methods of handling the picrate are well reviewed by A. P. Munch and R. T. Heukers<sup>16</sup> and W. L. Miller.<sup>17</sup> A new procedure has been proposed by A. Bolliger,<sup>18</sup> who determines the picrates by extractive titration in chloroform with methylene-blue.

For cyclopentadiene and its dimer, a colorimetric method has been described by K. Uhrig, E. Lynch, and H. C. Becker,<sup>19</sup> dependent on the

<sup>13</sup> *Zavodskaya Lab.*, 1945, **11**, 537, through *Chem. Abs.*, 1946, **40**, 2419.

<sup>14</sup> *Ind. Eng. Chem. (Anal.)*, 1944, **16**, 751

<sup>15</sup> *Chem.-Ztg.*, 1941, **65**, 446; 1943, **67**, 121. <sup>16</sup> *Chem. Weekblad*, 1935, **32**, 411.

<sup>17</sup> *J. Assoc. Off. Agric. Chem.*, 1934, **17**, 308.

<sup>18</sup> *Quart. J. Pharm.*, 1940, **13**, 1; *Analyst*, 1939, **64**, 416.

<sup>19</sup> *Ind. Eng. Chem. (Anal.)*, 1946, **18**, 550.

formation of the highly coloured phenylfulvene by condensation of *cyclopentadiene* with benzaldehyde. *Dicyclopentadiene* does not react, but is determined as additional monomer after controlled depolymerisation in the presence of decahydronaphthalene. It is stated that the materials normally associated with *cyclopentadiene* do not interfere; and though the method is devised with reference primarily to petroleum oils, it would appear potentially applicable to coal-tar spirits after a sufficient preliminary fractionation. R. Sefton<sup>20</sup> estimates *cyclopentadiene* in the lightest coal-tar distillates by measurement of its heat of condensation with maleic anhydride. The lack of specificity of the reaction is largely palliated by the much greater (almost instantaneous) speed of reaction with *cyclopentadiene*, as compared with the other unsaturated hydrocarbons likely to be present.

A second application of calorimetric technique is the estimation of small amounts of benzene in solution by measurement of its heat of nitration.<sup>21</sup> This method, though obviously limited in its scope, is said to be rapid and convenient for repeated estimates in special cases.

The gravimetric method for indene proposed by M. Weger and A. Billman,<sup>22</sup> based on precipitation of its benzylic compound on treatment with benzaldehyde, can be effectively applied to determination of indene in coal-tar fractions such as heavy naphtha, if these are first subjected to steam-distillation.<sup>23</sup>

The Höchst gravimetric procedure for anthracene in anthracene oils (oxidation to anthraquinone) has been adversely criticised by Cropper and Strafford,<sup>9</sup> who prefer the measurement of anthracene by ultra-violet absorption, after a chromatographic separation in which the anthracene zone on the chromatogram is located by its fluorescence in ultra-violet light. The ultra-violet fluorescence spectrum has been applied also to the evaluation of 1 : 2-benzpyrene in anthracene oils,<sup>24</sup> the anthracene being first eliminated by precipitating, from benzene solution, its complex with maleic anhydride.

These procedures are representative of a general tendency to supplement the limited number of chemical methods available for hydrocarbon analysis by physical, and especially by absorptiometric, techniques. Ultra-violet absorption has been applied to the analysis of benzene, toluene, and xylene mixtures by A. Luszczak<sup>25</sup> and P. Laurin,<sup>26</sup> who estimated the proportions of these compounds by comparing the intensities of those bands which are common to their spectra and those which are not. Characteristic bands, the intensity of which could be used as a measure of concentration, were reported by C. Weizmann, V. Henri, and E. Bergmann<sup>27</sup> for benzene, toluene, xylene, naphthalene, anthracene, and phenanthrene; and R. Schnurmann and

<sup>20</sup> *J. Soc. Chem. Ind.*, 1945, **64**, 104; see also A. V. Kirsanov, I. M. Polyakova, and Z. I. Kuznetsova, *J. Appl. Chem. U.S.S.R.*, 1940, **13**, 1406 (through *Chem. Abs.*, 1941, **35**, 2445) for iodometric estimation of the excess of maleic acid.

<sup>21</sup> R. L. Bishop and E. L. Wallace, *Ind. Eng. Chem. (Anal.)*, 1943, **15**, 563.

<sup>22</sup> *Ber.*, 1903, **36**, 640.

<sup>23</sup> R. D. Haworth, unpublished.

<sup>24</sup> A. Kling and M. Heros, *Compt. rend.*, 1941, **212**, 348.

<sup>25</sup> *Wien. Med. Wochenschr.*, 1936, **86**, 91, 150.

<sup>26</sup> *J. Pharm. Chim.*, 1938, **27**, 561.

<sup>27</sup> *Nature*, 1940, **146**, 230.

S. Whincup<sup>28</sup> have recorded the spectra of ethyl- and propyl-benzenes, styrene, and chrysene as well as the commoner aromatics. A. Berton<sup>29</sup> finds the bands narrower and more easily identified in the vapour state than in liquids, and has thus determined as little as 0.01 mg. of benzene per litre of air; similarly 0.1 mg./litre of toluene or styrene, and 0.2 mg./litre of xylene, anthracene, or phenanthrene.

R. R. Gordon and H. Powell<sup>30</sup> measure the optical density and molecular extinction coefficient of hydrocarbon mixtures (benzene and toluene, ethylbenzene and xylenes), obtaining readings at as many wave-lengths as the mixture has components. The total optical density at any wave-length being the sum of those of the individual components, the group of readings can be formulated as simultaneous equations and solved for the concentration of each component. Ultra-violet spectrophotometry applied to another hydrocarbon (diphenyl) is further mentioned below.<sup>38</sup>

Similar methods based on infra-red absorption have been applied to mixtures of benzene, toluene, and xylene with paraffins and naphthenes by B. Manière,<sup>31</sup> and although applications to benzenoid hydrocarbons have been worked out in less detail than those of ultra-violet spectroscopy, the development of analogous methods for petroleum oils<sup>32</sup> suggests that the infra-red may prove of similar use in the analysis of coal-tar spirits.

Use has also been made of the Raman spectra of the benzene hydrocarbons for their estimation in admixture with one another. Although P. Traynard<sup>33</sup> and D. H. Rank, R. W. Scott, and M. R. Fenske<sup>34</sup> were able to extend this technique only to binary mixtures (benzene and toluene), it has more recently<sup>35</sup> been applied to mixtures of from two to eight aromatic components, the proportions of which were determined with an accuracy of about 2%.

Spectroscopic methods have been similarly adopted to some extent for the analysis of the phenolic fractions from coal-tar. For instance, the quantitative and qualitative investigation, by infra-red absorption, of cresylic acids containing the three isomeric cresols has been described in papers by H. W. Thompson and D. H. Whiffen<sup>36</sup> and H. W. Thompson;<sup>37</sup> in the latter paper, the theoretical bases of the procedure are discussed in some detail. It is claimed that the bands of the infra-red spectra are in general sharper than those of the ultra-violet. By utilising the sharper bands obtained in the vapour as compared with the liquid state, however, A. Berton<sup>29</sup> has employed ultra-violet absorption for the determination of phenol and the three cresols. Mention may also be made of the estimation of *o*-hydroxydiphenyl, when used as a fungicide, by means of its ultra-violet absorption in *cyclohexane* solution.<sup>38</sup>

<sup>28</sup> *Petroleum*, 1945, **8**, 122.

<sup>29</sup> *Ann. Chim.*, 1944, **19**, 394.

<sup>30</sup> *J. Inst. Petroleum*, 1945, **31**, 428.

<sup>31</sup> *Ann. Chim. anal.*, 1941, **23**, 173.

<sup>32</sup> *E.g.*, D. L. Fry, R. E. Nusbaum, and H. M. Randall, *J. Appl. Physics*, 1946, **17**, 150.

<sup>33</sup> *Bull. Soc. chim.*, 1944, **11**, 552.

<sup>34</sup> *Ind. Eng. Chem. (Anal.)*, 1942, **14**, 816.

<sup>35</sup> D. H. Rank and R. V. Wiegand, *J. Opt. Soc. Amer.*, 1946, **36**, 325.

<sup>36</sup> *Chem. and Ind.*, 1944, 343.

<sup>37</sup> *Analyst*, 1945, **70**, 443.

<sup>38</sup> H. E. Cox, *ibid.*, p. 373. *cycloHexane* for spectroscopy may be freed from aromatics by filtration through silica gel (S. A. Ashmore, unpublished).



Diphenyl, which is generally present with its hydroxy-derivative, has also a well-defined maximum absorption, and can be similarly determined. In the case of *o*-hydroxydiphenyl, the use of a spectrophotometer is not indispensable, since the intensity of its fluorescence in ultra-violet light can be observed directly and compared with standards.

The chromatography of the phenolic compounds has been studied by W. Bielenberg and L. Fischer,<sup>39</sup> who concluded that direct adsorption of these constituents gave no promising results. After a preliminary coupling with diazotised *p*-nitroaniline, however, adsorption on alumina allowed of good separation; this procedure was utilised for qualitative identification of phenol, the three cresols, and *p*-xylenol in the presence of each other, and has subsequently been extended to all the hydroxy-benzenes boiling below 220°.

Although a number of methods for the chemical determination of phenols in particular circumstances have been published during the period under survey, yet most of these cannot be said to introduce any new chemical principle which might serve as a basis for a more general analytical reaction. Reference may be made to turbidity methods,<sup>40, 41, 42</sup> and to gravimetric methods with iodine—applied also to the naphthols and guaiacol<sup>43</sup> and to *o*-cresol and *o*-hydroxydiphenyl.<sup>44</sup> L. Bettelheim<sup>45</sup> separates phenol from phenol-cresol mixtures and higher homologues by shaking it out with a 33% solution of sodium benzenesulphonate, while J. N. Miller and O. M. Urbain<sup>46</sup> effect its differential oxidation with chromic acid; the total phenols being determined colorimetrically with diazotised sulphanilic acid both before and after this treatment, phenol may be estimated by difference.

The Chapin method for phenol, adopted by the Standardization of Tar Products Tests Committee, has been extended by T. S. Harrison,<sup>47</sup> using the Spekker absorptiometer, to the simultaneous estimation of *m*-cresol; the *o*- and *p*-isomers give no coloration with Millon's reagent, but *m*-cresol gives a yellow distinct from the red phenol colour, so that both can be determined in the same alkaline extract. In conjunction with the S.T.P.T.C. method for *o*-cresol (observation of the melting point of its complex with cineole<sup>48</sup>), C. E. Sage and H. R. Fleck<sup>49</sup> propose to utilise gravimetrically the resin-forming reaction given by *o*- and *m*-cresols, but not by *p*-cresol, with formaldehyde in acid solution; from the two analyses, the *m*-cresol content of

<sup>39</sup> *Brennstoff-Chem.*, 1940, **21**, 236; 1941, **22**, 278; 1942, **23**, 283.

<sup>40</sup> W. Seaman, A. R. Norton, and R. T. Foley, *Ind. Eng. Chem. (Anal.)*, 1943, **15**, 159.

<sup>41</sup> R. Paris and J. Vial, *Compt. rend.*, 1946, **222**, 324.

<sup>42</sup> J. Kay and P. J. C. Haywood, *Ind. Eng. Chem. (Anal.)*, 1944, **16**, 772.

<sup>43</sup> M. François and M. Seguin, *Bull. Soc. chim.*, 1933, **53**—**54**, 711.

<sup>44</sup> W. O. Emery and H. C. Fuller, *Ind. Eng. Chem. (Anal.)*, 1935, **7**, 248.

<sup>45</sup> *Svensk Kem. Tidskr.*, 1942, **54**, 194, 219, through *Chem. Abs.*, 1944, **38**, 3220, 3928.

<sup>46</sup> *Ind. Eng. Chem. (Anal.)*, 1930, **2**, 123. <sup>47</sup> *J. Soc. Chem. Ind.*, 1943, **62**, 119.

<sup>48</sup> F. M. Potter and H. B. Williams, *Analyst*, 1932, **57**, 267; 1939, **63**, 621.

<sup>49</sup> *Ibid.*, 1932, **57**, 567, 773.

mixed isomers can be estimated by difference. The resin-forming reaction with formaldehyde has also been advocated by A. Castiglioni<sup>50</sup> for the estimation of the naphthols; the method is applicable to either  $\alpha$ - or  $\beta$ -naphthol, but is not suitable for mixtures of the two. An alternative to the gravimetric treatment is colorimetric estimation of the  $\alpha$ -naphthol by means of the blue colour given by the  $\alpha$ -naphthol resin with sodium hydroxide.

The well-known Koppeschaar determination of phenols, by bromination and final titration of the iodine liberated from potassium iodide by the excess bromine, has been further studied by W. Bielenberg and E. Kuhn.<sup>51</sup> The course of the bromination was followed by a potentiometric method, and modifications of the standard technique, especially as regards the use of potassium iodide, are proposed.

For qualitative identification of many of the amino-compounds of the coal-tar distillates, the formation of characteristic diliturates (5-nitrobarbiturates)<sup>52</sup> may prove useful. The optical and crystallographic properties of crystals of these derivatives are distinctive even when prepared from mixtures of isomers.

Specific quantitative methods available in the amino-compound group are not very numerous. The estimation of residual free aniline in aniline derivatives is, however, often of importance, and an interesting technique for aniline in aminoazobenzene has been described by F. R. Cropper.<sup>53</sup> After diazotisation of the material and coupling with H-acid, the red dye produced from the aniline present is separated as a chromatogram on filter-paper, and the intensity of the band may be used as a quantitative measure of the aniline content. Aniline in methylanilines can be estimated by the picryl chloride method in ethyl acetate solution.<sup>54</sup> The sodium chloride resulting from neutralisation of the hydrochloric acid liberated is extracted with water and titrated potentiometrically against silver nitrate. An alternative manipulation in the picryl chloride method is described by G. Spencer and J. E. Brimley.<sup>55</sup>

A specific method for *p*-toluidine is based by C. H. Benbrook and R. H. Kienle<sup>56</sup> on the evolution of nitrogen on heating the diazotised material. The *p*-toluenediazonium derivative is relatively so stable, that *o*- and *m*-toluidines, aniline, etc., may be eliminated by three hours' heating of the diazotised mixture, and any nitrogen subsequently evolved used as a measure of the *p*-toluidine content.

For the polynuclear amino-compounds, no methods new in principle have been introduced for a considerable time. The methods available for diphenylamine, with special reference to its estimation when used as a stabiliser in explosives, have been reviewed and compared by F. Ellington.<sup>57</sup> A color-

<sup>50</sup> *Z. anal. Chem.*, 1938, **113**, 428.

<sup>51</sup> *Ibid.*, 1943, **126**, 88.

<sup>52</sup> B. T. Dewey and E. M. Plein, *Ind. Eng. Chem. (Anal.)*, 1946, **18**, 515.

<sup>53</sup> *Analyst*, 1946, **71**, 265.

<sup>54</sup> J. Haslam and F. Sweeney, *ibid.*, 1945, **70**, 413.

<sup>55</sup> *J. Soc. Chem. Ind.*, 1945, **64**, 53.

<sup>56</sup> *Ind. Eng. Chem. (Anal.)*, 1942, **14**, 427.

<sup>57</sup> *Analyst*, 1946, **71**, 305.

metric method based on oxidation with potassium dichromate has been proposed by H. Barnes.<sup>58</sup>

Among the heterocyclic constituents of the coal-tar distillates, preponderant importance attaches to pyridine, and critical surveys of the methods available for its determination have been published by A. Hamer, R. Pomfret, and W. V. Stubbings,<sup>59</sup> R. P. Daroga and A. G. Pollard,<sup>60</sup> and C. Belcot.<sup>61</sup> The method finally adopted by Daroga and Pollard, suitable for small quantities, is a colorimetric measurement of the blue produced by the action of reducing agents on the pyridine complex precipitated by silicomolybdic acid, while Hamer, Pomfret, and Stubbings prefer to utilise the clearing-temperature of solutions of pyridine perchlorate.

Although a number of colorimetric estimations of indole have been described, these mostly have reference to its production in bacterial cultures; they are thus appropriate only to small amounts, and are frequently not very specific. Mention may, however, be made of the qualitative colour reaction with xanthhydrol, which is not given by skatole or apparently by any other indole-ring compound which is substituted in the  $\beta$ -position;<sup>62</sup> and of the most recent reviews of the determination with Ehrlich's (*p*-dimethylamino-benzaldehyde) reagent.<sup>63, 64</sup>

For detection of acridine, J. C. Baro Graf<sup>65</sup> has recommended the preparation of highly characteristic crystals of the silicotungstate, easily distinguishable under the microscope from those given by pyridine or quinoline bases, and obtainable at a dilution of one part of acridine in 70,000. The procedure has, however, been criticised by G. Kohn and I. M. Kolthoff.<sup>66</sup> The technique of extractive titration of picrates and picrolonates against methylene-blue, already referred to,<sup>18</sup> has been applied also to the determination of the acridine bases.

Colorimetric methods for thiophen, intended for its estimation in "pure benzole" and therefore adapted to very small quantities of thiophen, have been worked out by K. H. V. French.<sup>67</sup> The colour reactions utilised are those with isatin in the presence of ferric sulphate and sulphuric acid, and with alloxan in the presence of sulphuric acid. The latter is somewhat the less sensitive, but the colour is more stable and gives on the whole better precision in working. F. S. Fawcett and H. E. Rasmussen<sup>68</sup> have determined the constants of a highly purified sample of thiophen, which may be of use in the preparation of standards for the colorimetric procedure.

E. G. K.

<sup>58</sup> *Analyst*, 1944, **69**, 344.

<sup>59</sup> *Ibid.*, 1946, **71**, 419.

<sup>60</sup> *J. Soc. Chem. Ind.*, 1941, **60**, 207T.

<sup>61</sup> *Ann. Chim. anal.*, 1938, **20**, 173.

<sup>62</sup> W. R. Fearon, *Analyst*, 1944, **69**, 122.

<sup>63</sup> I. H. Chernoff, *Ind. Eng. Chem. (Anal.)*, 1940, **12**, 273.

<sup>64</sup> M. B. Jacobs and S. Pincus, *Science*, 1945, **102**, 204.

<sup>65</sup> *Anal. Asoc. Quím. Argentina*, 1942, **30**, 44, through *Chem. Abs.*, 1942, **36**, 5732.

<sup>66</sup> *J. Biol. Chem.*, 1943, **148**, 711.

<sup>67</sup> *J. Soc. Chem. Ind.*, 1946, **65**, 15.

<sup>68</sup> *J. Amer. Chem. Soc.*, 1945, **67**, 1705.

### 3. MASS SPECTRA.

At very low pressures a suitable ribbon-shaped stream of positively charged gaseous ions can be deflected in a carefully chosen electric or magnetic field, or both, so that ions of each value of mass/charge are brought to a separate focus. If these operations are conducted in a mass spectrograph, the series of foci is arranged to fall on a photographic plate and produces a series of lines, each of which corresponds with a different mass provided each ion carries only one electronic charge. An ion carrying a multiple electronic charge suffers a larger deflection, and may be focused on the same spot as an ion of lower mass carrying a single electronic charge. In a mass spectrometer arrangements are made to focus in turn ions of each value of mass/charge on a slit, behind which is a device for collecting and recording electric charge or ion current.

Early work in the field showed that positively charged gaseous ions travelling at right angles to an electric field are deflected along the direction of the field, but in the case of a magnetic field deflection is at right angles to the field and to the line of motion.

When a pencil of positive ions all of equal mass and electronic charge and travelling with different velocities is subjected to parallel electric and magnetic fields at right angles to the line of motion, the ions fall on a parabolic curve on a plane perpendicular to the original line of motion. This is the principle of the parabola method described by (Sir) J. J. Thomson for the study of positive ions. He pointed out <sup>1</sup> that under these conditions ions of each different mass yield a different parabola, and therefore it would be possible to identify ions in terms of their masses, and indicated the value of the method in chemical analysis, including the fact that only a very small amount of material is required. It is important to remember that electrically neutral atoms and molecules are not deflected in this way, and the preliminary but essential process of ionisation which the volatile matter must undergo usually causes some decomposition thereby altering the composition, and it is only within very recent years that it has been found possible to relate the composition of the mixture of ions to the composition of the original electrically neutral gas or vapour mixture, thus establishing a means of determining the composition of the latter by positive-ray analysis or mass spectra. Earlier work <sup>2</sup> was devoted almost entirely to the discovery and identification of isotopes and the determination of their masses and relative abundance. In the course of this "analysis of the elements" various types of instrument were developed, based on several different methods devised for focusing the positive ions. In Aston's type of instrument the beam of ions is heterogeneous with respect to velocity, and resolution and focusing are achieved by deflecting the ions electrically and then magnetically in the opposite direction. But the beam of positive ions can be made homogeneous in

<sup>1</sup> "Rays of Positive Electricity and their Applications to Chemical Analyses", 1921, p. 179.

<sup>2</sup> F. W. Aston, "Mass Spectra and Isotopes", 1942.

respect of one of the possible variables before passing through the focusing fields, thereby permitting the complexity of the latter to be diminished. In K. T. Bainbridge's system,<sup>3</sup> the ions are passed through a "velocity selector" and all ions emerging have the same velocity and are deflected along semicircular paths and focused in a uniform magnetic field. W. R. Smythe and J. Mattauch<sup>4</sup> removed all ions save those having certain velocities by applying suitably spaced alternating electric fields at right angles to the beam of positive ions. The ions were then analysed by a radial electric field alone. Other developments including automatic recording are described by W. Bleakney and others.<sup>7</sup>

A mass spectrograph is used in the accurate determination of atomic masses since it is possible very accurately to determine the relative positions of lines made by positive rays on a photographic plate, and relative abundances of the different atoms may be determined by photometry of these lines. A mass spectrometer is used in the accurate determination of relative abundances of ions, and instruments of the form due to A. O. Nier<sup>5</sup> based on that of A. J. Dempster<sup>6</sup> are used in this type of analytical work. The rays are formed by the controlled ionisation of a stream of the vapour of the element or compound or mixture under test, and the ions, of mass  $m$  and charge  $e$ , are drawn out of the vapour stream by a small voltage and then accelerated through two slits by a suitable applied potential  $E$ , focused magnetically round the semicircular analyser, and collected on a plate behind the slit, and the resulting ion current is measured by a valve-amplifying device.

The equation of the circular path of radius  $r$  traversed by the ions focused on the slit by the magnetic field  $H$  which is at right angles to the plane of the semicircle is

$$m/e = H^2 r^2 / 2E$$

Ions of different mass may be focused successively on the slit by varying the accelerating potential  $E$ .

The parts of the analyser are enclosed in a Pyrex container which permits effective baking and out-gasing, a most important advance which permits the elimination of contamination by traces which by their presence would vitiate the analyses of substances introduced into the apparatus.

J. E. Taylor<sup>8</sup> described a Nier type mass spectrometer suitable for routine isotope abundance measurements. He out-gased at 350° for 48 hours before a determination, but there remained a small background of masses 18 (H<sub>2</sub>O) and 28 (CO<sub>2</sub>). The abundance ratio <sup>13</sup>C/<sup>12</sup>C was determined with a probable error of 2% from abundance measurements at mass 46 (<sup>12</sup>C<sup>16</sup>O<sup>18</sup>O), 45 (<sup>13</sup>C<sup>16</sup>O<sub>2</sub> and <sup>12</sup>C<sup>16</sup>O<sup>17</sup>O), and 44 (<sup>12</sup>C<sup>16</sup>O<sub>2</sub>).

H. G. Thode, P. L. Graham, and J. A. Ziegler<sup>9</sup> describe a mass spectrometer for rapid determination of isotope abundance ratios with high accuracy.

<sup>3</sup> *Physical Rev.*, 1932, **39**, 847.

<sup>4</sup> *Ibid.*, 1932, **40**, 429.

<sup>5</sup> *Ibid.*, 1937, **52**, 933.

<sup>6</sup> *Ibid.*, 1918, **11**, 316.

<sup>7</sup> *Ibid.*, 1932, **40**, 496; 1934, **45**, 761; 1938, **53**, 531.

<sup>8</sup> *Rev. Sci. Instr.*, 1944, **15**, 1.

<sup>9</sup> *Canadian J. Res.*, 1945, **23**, B, 40.

In a rapid recording instrument,<sup>10</sup> the mass spectrum is scanned across the exit slit by varying the magnetic field, a procedure favoured by N. D. Coggeshall.<sup>11</sup> Instruments are also the subject of patents.<sup>12</sup>

*Application.*—There are two fields of analytical chemistry in which mass spectrometry is an important, if not essential, technique. One is in the determination of isotope abundance ratios, and the other is in the analysis of mixtures of gases or vapours, particularly hydrocarbons. The advantage of speed which the technique confers in petroleum refinery analysis and plant control is frequently referred to.

*Isotope abundance ratios.* A substance suitable for the direct determination of the isotope abundance ratio of a constituent atom, by introduction into a mass spectrometer, must be sufficiently volatile, should be well chosen as to the masses of the ions which it will yield and must be pure and free from substances which afford ions of masses which interfere.

In measuring the rate of isotope exchange reactions between gases,<sup>13a</sup> in testing theories of thermal diffusion of gases, and in following changes in abundance ratios consequent upon the operation of processes designed to separate isotopes, the mass spectrometer has played an essential part. Significant features of technique already mentioned are illustrated by the work of A. O. Nier,<sup>13b</sup> who showed that a concentration gradient of  $^{12}\text{CH}_4$  and  $^{13}\text{CH}_4$  is set up in methane enclosed in a vessel in which a temperature gradient exists. Samples of the methane were burned to carbon dioxide in excess of pure oxygen, and the carbon dioxide was purified by condensation in a liquid-air trap, any excess of oxygen or unburned methane being pumped off. Water vapour was later condensed out at about  $-78^\circ$ . The carbon dioxide was ionised by controlled electron impact and the ion currents due to  $^{13}\text{CO}_2$  (mass 45) and  $^{12}\text{CO}_2$  (mass 44) were recorded. The relative heights of the peaks for masses 45 and 44 were corrected for the 7% contribution to the 45 peak due to  $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ . Tests showed that the burning of the methane and the subsequent manipulation had a slight but insignificant effect on the  $^{13}\text{C}/^{12}\text{C}$  ratio. It was necessary to burn the methane and operate with the resulting carbon dioxide rather than to analyse the methane itself owing to the identity of the masses of the ions  $^{13}\text{CH}_4$  and  $^{16}\text{OH}$ ; the latter is derived from the traces of water which could not be eliminated from the apparatus.

H. G. Thode,<sup>14</sup> in a review of the applications of stable isotopes, points out the advantages of using the mass spectrometer in place of density measurements on water when determining the abundance ratio of oxygen isotopes in tracer experiments. In order to avoid experimental difficulties which arise when  $^{18}\text{O}$  is introduced into a mass spectrometer in the form of water or

<sup>10</sup> J. A. Hipple, D. J. Grove, and W. H. Hickam, *Rev. Sci. Instr.*, 1945, **16**, 69.

<sup>11</sup> *J. Chem. Physics.*, 1944, **12**, 19.

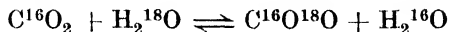
<sup>12</sup> H. Hoover, jun., U.S.P. 2,341,551, 15.2.44.

<sup>13a</sup> J. D. Brandner and H. C. Urey, *J. Chem. Physics*, 1945, **13**, 351.

<sup>13b</sup> *Physical Rev.*, 1939, **56**, 1009.

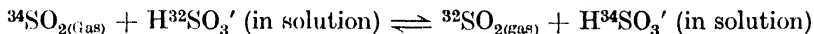
<sup>14</sup> *Canad. Chem.*, 1943, **27**, 647.

oxygen gas, M. Cohn and H. C. Urey<sup>15</sup> converted  $^{18}\text{O}$  into  $\text{C}^{16}\text{O}^{18}\text{O}$  by means of the exchange reaction



and determined the  $^{18}\text{O}/^{16}\text{O}$  ratio in the carbon dioxide obtained.

Such equilibria as



have been investigated extensively, and equilibrium constants can be calculated from the small differences in isotope abundance ratios found by mass spectrometer measurements on the relevant molecules in equilibrium in the vapour and liquid phases.<sup>9</sup>

The determination of nickel isotopes after diffusion of the stable isotopes of nickel into copper involved an elaborate series of processes preliminary to converting the nickel into nickel carbonyl which was analysed in a mass spectrometer.<sup>16</sup>

The versatility and fundamental importance of the technique is further illustrated by the accurate determination of differences in the abundance of lead isotopes 204, 206, 207, and 208, in common lead derived from minerals of various geological ages and in radiogenic leads. The metal was converted into lead iodide, and the vapour at about  $400^\circ$  and  $10^{-4}$ — $10^{-5}$  mm. Hg was ionised and analysed in a mass spectrometer.<sup>17</sup> From a consideration of these results it is concluded that the most probable age of the earth is 3,350 million years.<sup>18</sup>

*Non-radioactive tracer elements.* In principle, any element or atom in a molecule, with an isotope abundance ratio different from normal, can be followed through a sequence of processes or reactions by means of appropriate isotope abundance measurements. The analytical problems are very similar to those already indicated. Briefly, it may be necessary to synthesise the substance under investigation so that certain atoms have abnormal isotope abundance ratios and these are determined by converting a few mg. of the substance into molecules suitable for examination in a mass spectrometer. After completion of the processes under investigation, the products are isolated, purified, and converted into substances for isotope abundance ratio determination. For example, methionine was synthesised<sup>19</sup> to have isotope abundances above normal as indicated:  $\text{CH}_3\cdot^{34}\text{S}\cdot^{13}\text{CH}_2\cdot^{13}\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{CO}_2\text{H}$ , and when this was fed to rats, the cystine recovered from their hair had an abundance of  $^{34}\text{S}$  above normal, but the  $^{13}\text{C}/^{12}\text{C}$  ratio was normal.<sup>20</sup> The

<sup>15</sup> *J. Amer. Chem. Soc.*, 1938, **60**, 679.

<sup>16</sup> W. A. Johnson, *Amer. Inst. Min., Met. Eng.*, 1946, Tech. Publ. 2007, 13 pp.; *Metals Tech.*, **13**, No. 4.

<sup>17</sup> A. O. Nier, *J. Amer. Chem. Soc.*, 1938, **60**, 1571; A. O. Nier, R. W. Thompson, and B. F. Murphy, *Physical Rev.*, 1941, **60**, 112.

<sup>18</sup> A. Holmes, *Nature*, 1947, **159**, 127.

<sup>19</sup> G. W. Kilmer and V. du Vigneaud, *J. Biol. Chem.*, 1944, **154**, 247.

<sup>20</sup> V. du Vigneaud, G. W. Kilmer, J. R. Rachele, and M. Cohn, *ibid.*, **155**, 645.

carbon was examined isotopically as carbon dioxide, but it was more convenient to convert the sulphur into hydrogen sulphide than into sulphur dioxide<sup>21</sup> for examination.

Many valuable tracer experiments have been described during recent years<sup>22, 23</sup> which illustrate the application of the above-mentioned principles. The interpretation of the isotope ratios found may be complex matters,<sup>24</sup> and the possible incidence of isotope exchange reactions during the course of an investigation has to be considered.<sup>25</sup>

The "isotope dilution method" of analysis depends for its success on the provisos that a compound which has an abnormal isotope content of one or more elements is inseparable from the compound of normal isotopic composition by the ordinary laboratory procedures for isolating and purifying the compound,<sup>26</sup> and that the relevant atoms are found not to undergo exchange reactions during the various processes.<sup>27</sup> The method is particularly valuable in instances where it is difficult, if not impossible, to separate for determination in a pure form all of a constituent from a mixture; one may cite the difficulty of determining palmitic acid, for example, in a mixture of higher fatty acids, and the amino-acids in protein hydrolysates. The principle of the method is as follows.

To a weight of a mixture containing an unknown weight  $y$  of a substance Y is added a weight  $x$  of substance Y containing a concentration  $C_0$  above normal of, say, the heavy isotope of nitrogen,  $^{15}\text{N}$ , and after the mixture has been made homogeneous, a proportion of Y (it does not matter how small) is isolated and purified, and the concentration,  $C$ , of  $^{15}\text{N}$  above normal is determined; then  $y = (C_0/C - 1)/x$ .

The relative abundance of  $^{15}\text{N}$  in organic compounds has been determined<sup>28</sup> by digesting a weight of sample, containing 0.5—2 mg. of nitrogen, in a micro-Kjeldahl, and from the ammonia produced, the nitrogen is liberated by hypobromite and purified by passage through a trap immersed in liquid nitrogen. The ratio  $^{15}\text{N} : ^{14}\text{N}$  is determined with a precision of 0.003% in  $^{15}\text{N}$  in about 0.5 c.c. of the purified nitrogen which is introduced into a mass spectrometer, in which the ion currents due to  $^{14}\text{N}_2$  (mass 28) and  $^{15}\text{N}^{14}\text{N}$  (mass 29) are compared.

The application of the method to the determination of a particular compound involves the synthesis of that compound from substances in which the proportion of the rarer isotope of one of the elements has been increased, e.g.,  $^{15}\text{N}$  in ammonium salts,  $^{13}\text{C}$  in sodium cyanide, and  $^{34}\text{S}$  in sodium sulphate.

<sup>21</sup> A. O. Nier, *Physical Rev.*, 1938, **53**, 282.

<sup>22</sup> D. Rittenberg, *J. Appl. Physics*, 1942, **13**, 561.

<sup>23</sup> Many authors, *J. Biol. Chem.*, 1939, **130**, to 1946, **166**.

<sup>24</sup> E.g., D. Shemin and D. Rittenberg, *ibid.*, 1946, **166**, 621.

<sup>25</sup> H. G. Wood, C. H. Werkman, A. Hemingway, and A. O. Nier, *ibid.*, 1941, **139**, 377.

<sup>26</sup> D. Rittenberg and G. L. Foster, *ibid.*, 1940, **133**, 737.

<sup>27</sup> A. S. Keston, D. Rittenberg, and R. Schoenheimer *ibid.*, 1939, **127**, 315.

<sup>28</sup> D. Rittenberg, A. S. Keston, F. Rosebury, and R. Schoenheimer, *ibid.*, 1939, **127**, 291.



Synthetic methods may have to be devised to avoid any loss of the valuable rare isotope concentrate.<sup>29</sup>

Complications which are introduced by the use of synthetic isotope-rich racemic compounds with optically active substances in the ordinary way are eliminated by either racemising the optically active substances or by resolving the isotope-rich racemic compounds and using the appropriate isomers or by adding the racemic mixture and isolating the natural isomer. A modified procedure is used in attempting to detect *D*-glutamic acid in the presence of *L*-glutamic acid.<sup>30</sup> The accuracy of the method depends upon (1) the purity of the isotope-rich compound added, (2) the purity of the compound isolated (a point which can be checked by isotope ratio determination at successive stages of the determination), and (3) the accuracy of the isotope determination. The error due to the last may amount to about 1.5%.

The determination of the amino-acid composition of proteins is undergoing substantial advances<sup>31</sup> as regards both decrease in quantity of protein required and increased accuracy, and the isotope dilution method is playing a significant part. By the latter method, G. L. Foster<sup>32</sup> has determined the glutamic acid, aspartic acid, lysine, leucine, and glycine in only 7 g. of  $\alpha$ -lactoglobulin, the compounds isolated for purification being benzoylglycine, dibenzoyl-lysine, and the benzenesulphonyl derivative of leucine.

*Analysis of Mixtures.*—In principle it is possible to identify every element in a mixture by means of determinations of the masses of the isotopes present, but on the experimental side there are difficulties presented by the necessity of volatilizing the elements and, in some instances, of distinguishing between isotopes and molecular ions of equal mass. Moreover, a particular method of analysis, however excellent, will only be used or gain acceptance if it has advantages over existing methods.

The mass spectrometer has been applied to the analysis of traces of gases in mixtures of oxygen, nitrogen, and hydrogen, and of helium in nitrogen. It is also employed where less than 1 c.c. of gas is available, and where continuous indication of composition is desired.<sup>33</sup> The sensitivity of mass spectrum technique, used in conjunction with methods of concentrating material, in testing for the presence of traces may be illustrated by the demonstration that stable <sup>3</sup>H does not exist to anything like the extent of 1 in 10<sup>10</sup> in ordinary hydrogen.<sup>34</sup>

The composition of a mixture may be deduced from the relative proportions of the different elements found by means of a mass spectrometer; *e.g.*, hydrogen, helium, oxygen, nitrogen, neon, and argon have been determined in natural gas.<sup>50</sup> Interference by other ions was allowed for by selecting suitable mass peaks for observation.

<sup>29</sup> R. Schoenheimer and S. Ratner, *J. Biol. Chem.*, 1939, **127**, 301.

<sup>30</sup> S. Graff, D. Rittenberg, and G. L. Foster, *ibid.*, 1940, **137**, 745.

<sup>31</sup> *Ann. Reports*, 1945, **42**, 247.

<sup>32</sup> *J. Biol. Chem.*, 1945, **159**, 431.

<sup>33</sup> J. A. Hipple, *J. Appl. Physics*, 1942, **13**, 551.

<sup>34</sup> Lord Rutherford, *Nature*, 1937, **140**, 303; F. W. Aston, *Proc. Roy. Soc.*, 1937, **A**, **163**, 391.

*Compounds.* Apart from a few isolated instances, positive-ray or mass spectrum technique has not been applied to the analysis of mixtures of compounds until recently. It was thought that the method had shown the presence of methane, ethane, propane, and butane in the product obtained by irradiating with ultra-violet light a mixture of ethylene and hydrogen containing mercury vapour,<sup>35</sup> but subsequent experiments<sup>36</sup> with a mass spectrometer showed that under electron impact butane yields positive ions containing  $C_4$ ,  $C_3$ ,  $C_2$ , and  $C$ , and the propane previously reported might have been derived from butane disrupted by electron impact. It was found that the number and proportion of ionized fragments obtained by electron bombardment of various vapours depended on the nature of the molecule. Thus, approximately 60–80% of the positive ions derived from benzene contained  $C_6$ , 10–20% contained  $C_4$ , and small proportions only contained  $C_5$ ,  $C_3$ ,  $C_2$  and  $C$ .<sup>37</sup> Somewhat similar results were obtained by E. Friedländer and H. Kallmann.<sup>38</sup> These data were consistent with the proportions of individual ions  $C_6H_6$ ,  $C_6H_5$ ,  $C_6H_4$ , etc., found later<sup>39</sup> by means of mass spectrometers improved by developments in high-vacuum technique and the incorporation of arrangements for preventing contamination of the ion beam with products from the decomposition of the vapour at the source of electrons. Under similar conditions, octane was more extensively disrupted than benzene.<sup>40</sup> Relative abundances of the positive ions produced by controlled electron bombardment of ammonia ( $N$ ,  $NH$ ,  $NH_2$ ,  $NH_3$ ,  $NH_4$ , and  $N_2$ ), hydrazine ( $N_2H$ ,  $N_2H_2$ ,  $N_2H_3$ ,  $N_2H_4$ , and  $N$  ions),<sup>41</sup> methane,<sup>42</sup> ethane,<sup>43</sup> ethylene,<sup>44</sup> and methyl and ethyl alcohols<sup>45</sup> have been determined under a variety of conditions. The proportion of  $C_2H_5$  ions produced in the ionisation of *n*-butane is greater than in that of *isobutane*<sup>46</sup> and this is evidently related to differences in the dissociation probabilities of the different linkings under electron impact.<sup>47</sup>

As a sequel to and consistent with these results, it is found that with modern technique each hydrocarbon, methane, ethane, etc., gives its own particular abundance ratio of the various ions into which it is broken down by controlled ionisation. These characteristic abundance ratios are obtained

<sup>35</sup> A. R. Olson and C. H. Meger, *J. Amer. Chem. Soc.*, 1927, **49**, 3131.

<sup>36</sup> H. R. Stewart and A. R. Olson, *ibid.*, 1931, **53**, 1236.

<sup>37</sup> E. G. Linder, *Physical Rev.*, 1932, **41**, 149.

<sup>38</sup> Z. *physikal. Chem.*, 1932, *B*, **17**, 265.

<sup>39</sup> P. Kusch, J. T. Tate, and A. Hustrulid, *Physical Rev.*, 1937, **51**, 1007; 1938, **54**, 1037.

<sup>40</sup> E. G. Linder, *J. Chem. Physics*, 1933, **1**, 129.

<sup>41</sup> D. D. Taylor, *Physical Rev.*, 1935, **47**, 666.

<sup>42</sup> J. A. Hipple, jun., and W. Bleakney, *ibid.*, p. 802.

<sup>43</sup> J. A. Hipple, jun., *ibid.*, 1938, **53**, 530.

<sup>44</sup> P. Kusch, A. Hustrulid, and J. T. Tate, *ibid.*, 1937, **52**, 843.

<sup>45</sup> C. S. Cummings and W. Bleakney, *ibid.*, 1940, **58**, 787.

<sup>46</sup> R. F. Baker and J. T. Tate, *ibid.*, 1938, **53**, 944.

<sup>47</sup> J. Delfosse and J. A. Hipple, jun., *ibid.*, **54**, 1060; M. W. Evans, N. Bauer, and J. Y. Beach, *J. Chem. Physics*, 1946, **14**, 701.

irrespective of whether the substance is pure or in a mixture. Further, if each of two or more constituents of a mixture yields a common ion, the proportion of this ion measured is the sum of the proportions derived from each of the constituents. Isomers, such as *n*- and *iso*-butane, yield different abundance ratios, and as a consequence isomers can be determined in mixtures.<sup>47a</sup>

H. W. Washburn, H. F. Wiley, and S. M. Rock<sup>48</sup> used a Nier type instrument; ions of each mass were caused to fall successively on the collector and the resulting successive ion currents were so amplified that the magnitude of each ion current, or peak, was recorded at four different amplifications to 1% on an oscillograph. The quantitative analysis of such mixtures as propylene, propane, *n*- and *iso*-butane, *isobutylene*, butylene-1, butylene-2, *n*- and *iso*-pentane, and pentenes was carried out relatively rapidly. Less than 0.1 c.c. of liquid sample was required and a complete analysis, including the computations involved, usually took less than 4 hours compared with several days required by other methods. Routine analysis of such mixtures required substantially less than 4 hours. The error in determining components present in large proportion was usually less than 1 mol. %, and in the case of constituents present in very small proportions was usually less than 10% of the mol. % actually present. As many as 20 samples, containing as many as 15 components, could be analysed in an 8-hour day. As an illustration of the method of computation employed, in a mixture of *n*- and *iso*-butane, propane, ethane, and methane, only the first two contributed to peaks at masses 57 and 58, and by means of coefficients derived from calibration experiments with the pure hydrocarbons, the proportions were calculated, and their contributions to the peak at mass 44 deducted from the observed value, the balance being due to propane. The ethane and methane contents were similarly deducted from values at masses 30 and 16 respectively. The contribution of each hydrocarbon to other mass peaks was calculated, and the analysis was regarded as satisfactory if the residuals were less than 1% of the peaks. Larger discrepancies were attributed to the presence of other substances and led, for example, to the detection and determination of acetone and naphthenes in two hydrocarbon mixtures. The technique has been used for the analysis of mixtures of aromatic hydrocarbons and for the determination of small amounts (0.036–8%) of diethylbenzene in ethylbenzene. Six isomeric octanes showed sufficient differences to permit the composition of mixtures to be determined with an error of less than  $\pm 1.7$  mol. %, but extension of the method to organic compounds containing oxygen has not met with uniform success.<sup>49</sup>

The method of analysis has been examined by A. K. Brewer and V. H. Dibeler,<sup>50</sup> who have identified and determined 10 impurities in 1:3-butadiene of 98% purity and analysed many mixtures of gases and vapours,

<sup>47a</sup> D. P. Stevenson and J. A. Hipple, jun., *J. Amer. Chem. Soc.*, 1942, **64**, 1588.

<sup>48</sup> *Ind. Eng. Chem. Anal.*, 1943, **15**, 541.

<sup>49</sup> H. W. Washburn, H. F. Wiley, S. M. Rock, and C. E. Berry, *ibid.*, 1945, **17**, 74.

<sup>50</sup> *J. Res. Nat. Bur. Stand.*, 1945, **35**, 125; 1946, **36**, 338.

including natural gas and oil-flame fumes with as many as 14 components. Duplicate analyses agreed to 0.1—0.001%. The vapour at about  $10^{-5}$  mm. Hg pressure is bombarded with electrons having not less than 50 volts of energy, and the ratios of the fragments into which the molecules are broken are the same over a wide range of pressure for each molecular species but are never the same for different species. Errors in the analysis may originate in the instrument, in the sample, or in the computation. In precision work, the mass spectra of the main ingredients of the mixtures should be checked daily. The proportion of ions with more than one electronic charge is small and can be allowed for. The heights of certain peaks are corrected by deducting contributions arising from the inclusion of ions containing  $^{13}\text{C}$  or D. Thus, the peak of mass 44 will include propyl ions containing either of these heavy isotopes, and corrections are calculated from adjacent lighter peaks by means of coefficients. A table of these correction factors is given. Accuracy in the preparation from pure components of small quantities of mixtures of known composition has been increased. R. C. Taylor and W. S. Young<sup>51</sup> describe the use of valves of sintered glass and mercury in mixing definite quantities of volatile liquids and in introducing the mixtures into mass spectrometers. The analysis, by mass spectrometer, of a standard mixture of six isomeric octanes prepared by this means was consistent with the proportions mixed. R. H. Busey, G. L. Barthauer, and A. V. Metler<sup>52</sup> blend low-boiling hydrocarbons by means of small bombs of the pure hydrocarbons connected to a system of measuring vessels, a manometer, and a stock bomb into which the pure hydrocarbons are successively condensed. Composition is calculated from individual pressure measurements or changes in the weight of the bombs.

J. G. A. G.

#### 4. INFRA-RED ABSORPTION SPECTRA.

Until recent years, the analytical applications of infra-red absorption spectroscopy have been limited mainly to the photographic and overtone vibration regions extending from  $0.75\ \mu$  to  $2.5\ \mu$  approximately.<sup>1</sup> Beyond the photographic region ( $0.75$ — $1.3\ \mu$ ) the mapping of spectra was tedious owing to the limitations of instruments, and relatively slow progress was made. The discovery of the unique characteristics of the infra-red absorption spectra of molecules has stimulated the development of technique; and the striking advances recently reported<sup>2</sup> extend the range of application to  $15\ \mu$  (the limit of transmission of rock-salt), and in principle to  $25\ \mu$  (the limit of transmission of potassium bromide). It appears that the fundamental vibration region, which covers the range  $2.5$ — $25\ \mu$  approximately, is providing data of great utility in analytical chemistry, and the large number of publications during the past year justify a short report which should be read against the background of last year's report on "Recent Advances in Infra-red Spectroscopy".<sup>2</sup>

<sup>51</sup> *Ind. Eng. Chem. Anal.*, 1945, **17**, 811.

<sup>52</sup> *Ibid.*, 1946, **18**, 407.

<sup>1</sup> *Ann. Reports*, 1931, **28**, 181; 1938, **35**, 395.

<sup>2</sup> *Ibid.*, 1945, **42**, 5.

It is convenient to recall that there are two units employed in designating portions of the infra-red spectrum: a wave-length unit, the micron,  $\mu$  ( $10,000 \text{ \AA.} = 1 \mu = 10^{-4} \text{ cm.}$ ), and a frequency unit, the wave-number or  $\text{cm.}^{-1}$ , related to the wave-length unit by  $1 \text{ cm.}^{-1} = 1/\lambda \text{ (cm.)}$ ; for example,  $4000 \text{ cm.}^{-1} = 1/2.5 \times 10^{-4} \text{ cm.} = 1/2.5 \mu$ .

Investigations of infra-red absorption spectra depend upon the availability of (1) a source emitting a continuous range of wave-lengths, (2) a means of focusing and dispersing this radiation into very narrow bands of definite wave-lengths, (3) a means of interposing a sample of suitable thickness in the path of the radiation, and (4) means of detecting, measuring, and recording the radiation transmitted.

The source commonly employed is a Nernst filament or a silicon carbide rod (Globar) electrically heated, but nichrome<sup>3</sup> and an alloy containing iron, chromium, and aluminium<sup>4</sup> have also been used. Owing to the difficulty of constructing achromatic and transparent lenses for focusing infra-red radiation, surface-coated mirrors of gold or aluminium<sup>5</sup> are employed. Prisms transparent to the limits indicated (glass  $1.5 \mu$ , quartz  $3 \mu$ , lithium fluoride  $5 \mu$ , fluorite  $9 \mu$ , rock-salt  $15 \mu$ , and potassium bromide  $25 \mu$ ) are generally used to disperse the radiation. In a fresh comparison of relative merits, fluorite is preferred to lithium fluoride.<sup>6</sup>

Calibration of a prism spectrometer may be effected by means of 6 or 8 points ranging from the sodium D line to the carbon dioxide  $14.97 \mu$  band.<sup>7</sup> Although atmospheric moisture causes deterioration of rock-salt surfaces, it is much used as a prism material and as the transparent portions of absorption cells. Resistance to atmospheric corrosion may be increased by heating at  $500^\circ$  for a few hours.<sup>8</sup> Other precautions, such as a small heating element under the prism tables, may be instituted.<sup>9</sup> Absorption cells for substances molten at elevated temperatures may be made by cementing rock-salt plates to "Pyrex" glass with silver chloride, but the cell must be kept above  $150^\circ$  to prevent stresses from cleaving the rock-salt.<sup>10</sup>

Advances in the detection, measurement, and recording of infra-red radiation are very striking. The present limit of photography ( $1.3 \mu$ ) is likely to be extended to  $1.53 \mu$  by placing infra-red sensitive phosphor screens in contact with photographic plates.<sup>11</sup> A sensitive photo-conductive cell of lead sulphide with maximum sensitivity at  $2.5 \mu$  and threshold at  $3.6 \mu$  is mentioned,<sup>12</sup> and filters of organic dyes and plastics, transmitting in the region  $1\text{--}3 \mu$  but passing little visible radiation, have been produced.<sup>13</sup>

<sup>3</sup> N. Wright and W. Herscher, *J. Opt. Soc. Amer.*, 1946, **36**, 195.

<sup>4</sup> J. Savage, *J. Sci. Instr.*, 1946, **23**, 295.

<sup>5</sup> N. W. Scott, *J. Opt. Soc. Amer.*, 1946, **36**, 711.

<sup>6</sup> R. C. Gore, R. S. Macdonald, V. Z. Williams, and J. U. White, *ibid.*, 1947, **37**, 23.

<sup>7</sup> D. S. McKinney and R. A. Friedel, *ibid.*, 1946, **36**, 715.

<sup>8</sup> A. Elliott, *Nature*, 1946, **157**, 299.     <sup>9</sup> P. J. Kipp, *J. Sci. Instr.*, 1946, **23**, 246.

<sup>10</sup> G. L. Simmard and J. Steger, *Rev. Sci. Instr.*, 1946, **17**, 156.

<sup>11</sup> F. W. Paul, *J. Opt. Soc. Amer.*, 1946, **36**, 175.     <sup>12</sup> R. J. Cashman, *ibid.*, p. 356.

<sup>13</sup> E. R. Blout, W. F. Amon, jun., R. G. Shepherd, jun., A. Thomas, C. D. West, and E. H. Land, *ibid.*, p. 460.

The rapid scanning and recording of absorption spectra demands radiation detectors of small time constant, and comparative studies of the performance of infra-red receivers have been made.<sup>14</sup>

In respect of speed of response, thermopiles are somewhat wanting, but the construction of thermopiles having time constants of only 0.01—0.03 sec.<sup>15</sup> suggests that the objection has been at least partly removed. Sensitive thermopiles of several designs, including vacuum types, have been described.<sup>16</sup> In one instrument, two halves may be connected in opposition giving a compensated thermopile which is free from drift or the two halves may be illuminated with different beams such as may be obtained with a compensated optical system. Another thermopile has four receivers in line for use with a double-beam infra-red spectrometer in which each of two different beams fall on one of the two inner receivers, the two outer receivers providing compensation. In some instruments the time constant has been diminished to less than 0.05 sec.

The construction of sensitive bolometers, instruments in which use is made of the rapid change of resistance of a metal ribbon or film with temperature, has been described.<sup>17</sup> The application of a fast superconducting instrument operating at 14° K. is foreshadowed.<sup>18</sup> Thermistor bolometers are made of semi-conductors of which the resistance varies rapidly with temperature. An instrument with a time constant of 3 milliseconds is described<sup>19</sup>, together with a detector system which scans 1  $\mu$  in 1 minute.<sup>20</sup>

G. F. Lothian<sup>21</sup> has given a survey of modern spectrometers, and, although the relative merits of single- and double-beam instruments are debated,<sup>22</sup> reference may be made to a mirror double monochromator intended for work in the infra-red, visible, and ultra-violet regions of the spectrum, and having two prisms each of quartz (0.2—2.7  $\mu$ ), flint (0.4—1.5  $\mu$ ), and rock-salt (0.2—16.0  $\mu$ ). The radiation receiver for infra-red is a compensated thermopile and galvanometer, deflections of which may be magnified by a relay outfit and secondary galvanometer.<sup>23</sup> J. U. White<sup>24</sup> described a simple infra-red spectrometer recording optical densities directly, and means of presenting spectra extending over widths as great as 3  $\mu$  on the screen of a cathode-ray tube have been reported.<sup>25</sup>

*Analysis.*—Theory and experiment appear to be in agreement that unless two molecules are identical, or are optical enantiomorphs, they will have

<sup>14</sup> E. E. Bell, R. F. Bahl, A. H. Nielsen, and H. H. Neilsen, *J. Opt. Soc. Amer.*, 1946, **36**, 355.

<sup>15</sup> L. Harris, *ibid.*, p. 597.

<sup>16</sup> E. Schwarz, *J. Sci. Instr.*, 1946, **23**, 246.

<sup>17</sup> F. G. Brockman, *J. Opt. Soc. Amer.*, 1946, **36**, 32; B. H. Billings, W. L. Hyde, and E. E. Barr, *ibid.*, p. 354.

<sup>18</sup> D. H. Andrews, R. M. Milton, and W. DeSorbo, *ibid.*, p. 518.

<sup>19</sup> W. H. Brattain and J. A. Becker, *ibid.*, p. 354.

<sup>20</sup> J. A. Becker and H. R. Moore, *ibid.*, p. 354.

<sup>21</sup> *J. Sci. Instr.*, 1946, **23**, 293.

<sup>22</sup> W. C. Price, *ibid.*, p. 295.

<sup>23</sup> P. J. Kipp, *ibid.*, p. 246.

<sup>24</sup> *J. Opt. Soc. Amer.*, 1946, **36**, 362.

<sup>25</sup> E. F. Daly and G. B. M. Sutherland, *Nature*, 1946, **157**, 547; J. King, R. B. Temple, and H. W. Thompson, *ibid.*, **153**, 196.

different arrays of vibration frequencies and correspondingly different infra-red absorption spectra. It follows that each pure substance has its own characteristic infra-red absorption spectrum by which it can be identified. In order to operate a system of identification based on these spectra, a large number need to be recorded and suitably classified. Spectra of 363 organic compounds have been indexed<sup>29</sup> and there are many distributed throughout the literature. As illustrative of the value of infra-red methods of identification, and foreshadowing applications in the analysis of plastics, it is found that natural rubber and synthetic rubbers afford different distinctive absorption spectra which can be used in the quantitative analysis of mixtures.<sup>26</sup> In an examination of 7 *cyclopentanes* and 5 *cyclohexanes*, the spectral differences between four dimethyl*cyclopentanes* were found to be quite marked.<sup>27</sup> *neo*Pentane, not found hitherto in crude oil, was identified and determined along with the other constituents of a fraction also containing propane, *n*- and *iso*-butane, and *isopentane*.<sup>28</sup>

There are, however, spectral similarities between chemically related substances, and, as a result of correlation work, it has been found that certain groups of atoms, and linkings, give rise to absorption bands in characteristic regions of the spectrum.<sup>29</sup> These depend in some measure on the masses of the vibrating parts, and in this connection it may be recalled that the existence of the heavy isotope of hydrogen was confirmed by the discovery of bands due to  $^2\text{H}^{35}\text{Cl}$  and  $^2\text{H}^{37}\text{Cl}$  in the calculated position about  $4.8\ \mu$  as compared with  $3.46\ \mu$  for  $^1\text{HCl}$ .<sup>30</sup> In the high-frequency ( $3\ \mu$ ) region, the stretching vibrations between hydrogen and other atoms give rise to bands between the limits indicated: O-H  $3700\text{--}3500\ \text{cm}^{-1}$  (if hydrogen bonding occurs, the frequency is lower), N-H  $3500\text{--}3200\ \text{cm}^{-1}$ , C-H  $3200\text{--}2800\ \text{cm}^{-1}$ , S-H  $2500\ \text{cm}^{-1}$  approximately; C:C, C:N, and C:O are related to an absorption at close to  $2000\ \text{cm}^{-1}$ ; C:O in esters, acids, aldehydes, and ketones affords absorption at  $1750\text{--}1650\ \text{cm}^{-1}$ , and aliphatic C:C at  $1660\text{--}1600\ \text{cm}^{-1}$ . However, absorptions such as that due to C:C may be weak or non-existent if the linking occurs in a symmetrical position in a molecule, since infra-red absorption only occurs if the associated vibration causes a change in dipole moment. Other correlations relating to more complex groupings have been worked out, and they all play an essential part in analysis. The unexpected appearance of such a band in the absorption spectrum of a substance of known spectrum indicates the presence of an impurity. For example, *isoborneol* in camphor may be detected down to low limits by the O-H band near  $2.9\ \mu$ .<sup>31</sup> If the chemical history of the substance is

<sup>26</sup> R. B. Barnes, V. Z. Williams, A. R. Davis, and P. Giesecke, *Ind. Eng. Chem. (Anal.)*, 1944, **16**, 9.

<sup>27</sup> E. K. Plyler, R. Stair, and C. J. Humphreys, *J. Opt. Soc. Amer.*, 1946, **36**, 716.

<sup>28</sup> L. C. Jones, jun., R. A. Friedel, and G. P. Hinds, jun., *Ind. Eng. Chem. (Anal.)*, 1945, **17**, 349.

<sup>29</sup> R. B. Barnes, R. C. Gore, U. Liddel, and V. Z. Williams, "Infra-red Spectroscopy", 1944.

<sup>30</sup> J. D. Hardy, E. F. Barker, and D. M. Dennison, *Physical Rev.*, 1932, **42**, 279.

<sup>31</sup> G. B. B. M. Sutherland, *Trans. Faraday Soc.*, 1945, **41**, 206.

known, the alien band may afford an important clue as to the identity of the impurity, particularly as the precise values of these frequencies are related to the structure of the rest of the molecule. When the identity of the impurity is established, then the characteristic band may also be used to determine the proportion present.

Some recent work on DDT [1 : 1 : 1-trichloro-2 : 2-di-(4-chlorophenyl)-ethane] brings out the advantages of using infra-red absorption as compared with other analytical methods such as the determination of halogen or colorimetric reactions.<sup>32</sup> DDT has strong bands at 9.1  $\mu$  and 9.8  $\mu$  which are common to isomers and impurities containing a *p*-Cl-substituted phenyl grouping; isomers and other impurities have bands not possessed by DDT as indicated :

- 1 : 1 : 1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane, 9.6  $\mu$  (*o*-chlorophenyl group) and 13.3  $\mu$ ;
- 1 : 1 : 1-trichloro-2-(3-chlorophenyl)-2-(4-chlorophenyl)ethane, 10.9  $\mu$ ;
- 1 : 1-dichloro-2 : 2-di-(4-chlorophenyl)ethylene, 10.2  $\mu$ ;
- di-4-chlorophenyl sulphone, 7.5, 8.6, and 7.8  $\mu$ ;
- 2 : 2 : 2-trichloro-1-(2-chlorophenyl)ethyl 4-chlorobenzenesulphonate, 8.4 and 10.1  $\mu$ .

The presence of these bands in commercial DDT was presumptive evidence of the presence of the corresponding impurities, and their concentrations could be inferred from absorption measurements at wave-lengths corresponding with these key bands. The fact that the absorption spectrum of *pp'*-DDD [1 : 1-dichloro-2 : 2-di-(4-chlorophenyl)ethane] in the range 7—15  $\mu$  shows only small differences from that of DDT adds a warning note that infra-red technique has limitations.

The failure to observe certain bands characteristic of impurities does not necessarily imply their absence. A certain minimum concentration, possibly several units %, may be necessary before bands from the impurity can be distinguished from absorption due to the principal substance, or bands due to the latter may mask those of the impurity. As instances, as little as 0.5% of ethyl alcohol can be detected in acetaldehyde by the alcohol band of wave-number 1052  $\text{cm}^{-1}$ . The O-H band was not used because the aldehyde absorbs at 2.9  $\mu$ .<sup>31</sup> Concentrations of  $\alpha$ -pinene as low as 2% could be detected in a mixture of terpenes by a band at 787  $\text{cm}^{-1}$ . Illustrative of the versatility of the technique, there have been detected organic phosphites in phosphonates, impurities in ethylidene chloride and tetrachloroethylene, trichlorobenzoic acid in the dichloro-acid,<sup>33</sup> cyclohexane in toluene, and as little as 1 p.p.m. of hexane in carbon tetrachloride.<sup>34</sup>

*Quantitative Analysis.*—Quantitative analysis may be performed by

<sup>32</sup> J. R. Downing, W. V. Freed, I. F. Walker, and G. D. Patterson, *Ind. Eng. Chem. (Anal.)*, 1946, **18**, 461.

<sup>33</sup> D. H. Wiffen, P. Torkington, and H. W. Thompson, *Trans. Faraday Soc.*, 1945, **41**, 200.

<sup>34</sup> R. C. Gore and J. B. Patberg, *Ind. Eng. Chem. (Anal.)*, 1941, **13**, 768.



empirical calibrations using mixtures of known composition, or by application of Beer's law, but it must be borne in mind that this law would be expected to be followed only if determinations of optical density are made with homogeneous radiation. If a narrow band of wave-lengths is employed, as is usual in infra-red work by reason of the finite slit width which has to be employed, then departures from Beer's law may be expected whenever the observations are made at wave-lengths over which the extinction coefficient  $\epsilon$  changes sharply with change of wave-length. In practice, observations made at the peaks of bands usually follow the Lambert-Beer law.

On the assumption that these laws are applicable to each of the  $n$  components of a mixture, and that the optical density,  $d_\lambda$  at any wave-length, is the sum of the optical densities of the components, then, if  $l$  is the thickness of the cell,

$$\begin{aligned} d_\lambda &= d_{1\lambda} + d_{2\lambda} + \dots + d_{n\lambda} \\ &= l(C_1\epsilon_{1\lambda} + C_2\epsilon_{2\lambda} + \dots + C_n\epsilon_{n\lambda}) \end{aligned}$$

Consequently, it is necessary to determine the optical density at each of  $n$  wave-lengths, at which the values of  $\epsilon$  have been determined for the pure components, in order to obtain  $n$  equations from which the concentrations  $C_1, C_2, \dots, C_n$  can be calculated. The calculations may be facilitated by special manipulation of the linear equations and the use of a calculating machine,<sup>35</sup> but the accuracy of the concentrations deduced depends upon several factors. For highest accuracy, the percentage absorptions should be in the neighbourhood of 63%; an error of 1% leads to larger errors in the value of  $C$ .<sup>36</sup> The thickness of absorption cells may be made as small as 0.005 mm. in consequence of high values of  $\epsilon$  exhibited by many liquids, and the accuracy with which values of  $C$  can be calculated depends on the accuracy with which  $l$  is determined. Interference methods with infra-red and visible light and the weight of contained liquid have been used.<sup>37</sup> J. H. Lee<sup>38</sup> has developed methods of correcting for (1) scattered energy which reaches the receiver by circuitous paths, (2) errors resulting from finite slit width and narrow absorption bands, (3) pressure broadening and the effects of admixed molecules, and has determined the composition of hydrocarbon vapour mixtures containing five components with an error of less than 1.5 mol.%. He records data relating to bands of 12 hydrocarbons. For instances of binary mixtures to which Beer's law does not apply, M. Fred and F. W. Porsche<sup>39</sup> describe a graphical method in which observed optical densities of the mixture determine the location of a point inside a co-ordinate network reading directly in concentration.

J. Lecomte<sup>40</sup> gives criteria for the use of infra-red spectra in determining the purity of hydrocarbons and makes the point that in the analysis of

<sup>35</sup> L. J. Comrie, *J. Sci. Instr.*, 1944, **21**, 129; J. L. Saunderson and H. H. Grossman, *J. Opt. Soc. Amer.*, 1946, **36**, 243.

<sup>36</sup> A. R. Philpotts, *Trans. Faraday Soc.*, 1945, **41**, 197.

<sup>37</sup> G. B. B. M. Sutherland and H. A. Willis, *ibid.*, p. 181; A. E. Martin, *ibid.*, p. 181.

<sup>38</sup> *Ind. Eng. Chem. (Anal.)*, 1946, **18**, 659.

<sup>39</sup> *Ibid.*, p. 603.

<sup>40</sup> *Compt. rend.*, 1946, **222**, 648.

hydrocarbon mixtures, such as motor spirits, the spectra are too complicated unless carefully purified fractions are employed. Preliminary separations are often an essential feature, as, for example, in the analysis of mixtures of xylenols.<sup>33</sup>

Infra-red absorption spectroscopy is playing a part in many branches of analytical chemistry and recent applications include the accurate spectrophotometric determination of copper in hydrochloric acid solution by means of measurements at  $0.97 \mu$ <sup>41</sup> and routine determination of  $\text{Cu}^{++}$ ,  $\text{Fe}^{++}$ ,  $\text{Ni}$ , and  $\text{Co}^{++}$  may be made by means of thallium sulphide photo-elements.<sup>12, 42</sup> Minute amounts of hydrocarbons in soil gases may be determined by combustion to carbon dioxide which is measured by infra-red absorption at  $2.5\text{--}2.8 \mu$ ,<sup>43</sup> and the water vapour in a vertical column of the atmosphere has been determined by using a transmission replica grating and an infra-red sensitive photo-cell to compare the radiant flux in the  $0.94 \mu$  water vapour absorption band with that at  $1.01 \mu$  where no absorption occurs.<sup>44</sup> The determination of leucine and isoleucine in mixtures of the two derived from the hydrolysis of proteins is a matter of great difficulty analytically, and G. B. B. M. Sutherland<sup>45</sup> has found sufficient differences between infra-red absorption spectra of these amino-acids, and also between their acetyl derivatives, to permit determinations of the proportions with an accuracy of about 5%. The concentrations of oxyhæmoglobin, methæmoglobin, and carboxyhæmoglobin in samples of blood have been determined from spectrophotometric observations made in the infra-red and the visible region of the spectrum.<sup>46</sup>

Among the advantages of infra-red absorption spectroscopy as a method of analysis, mention may be made of (1) the small quantities of material usually required for an analysis, (2) the absence of any decomposition and its consequences, by the radiation, except perhaps in very rare instances, and (3) the simplicity and speed with which an analysis can be performed after the preliminary calibrations have been made. Sufficient has been said to indicate that the technique, in common with other physical methods of chemical analysis, has its own particular fields of utility, in parts of which it is the method of choice, and in certain circumstances may be the only method by which it is possible to carry out a particular analysis. J. G. A. G.

## 5. THE DETERMINATION OF SMALL QUANTITIES.

It was the intention of the Reporter, under the above title, not only to review the progress made in the determination of small quantities, but also to use such a review to indicate the present trends in analytical chemistry.

<sup>41</sup> P. Giesecke, *Amer. Inst. Min. Met. Eng.*, 1944, Tech. Publ. 1740, 15 pp.; *Min. Tech.*, 8, No. 5.

<sup>42</sup> G. Berraz and E. Virasoro, *Anal. Inst. invest. cient. tecn.*, 1942—1943, 12—13, 147.

<sup>43</sup> W. J. Sweeney, U.S.P. 2,170,435, 22.8.39.

<sup>44</sup> N. B. Foster and L. W. Foskett, *J. Opt. Soc. Amer.*, 1945, 35, 601.

<sup>45</sup> *J. Inter. Soc. Leather Trades Chem.*, 1946, 30, 11.

<sup>46</sup> B. L. Horecker and F. S. Brackett, *J. Biol. Chem.*, 1944, 152, 669.

The term "small quantities" was to include, not only analyses made where the total material available was small, but also those where the quantity determined was small irrespective of the amount of material available. The review of work done was therefore to include largely microchemical methods, together with certain applications of quantitative spectrography, colorimetric and turbidity measurements, polarography, X-ray diffraction analysis, and some macro-analytical methods. A recent monograph<sup>1</sup> has, however, provided a very complete review of microchemical progress besides drawing attention to other reviews dealing with the same subject.<sup>2,3,4</sup> In these circumstances further detailed reference to such work here is unnecessary, and the Reporter intends to proceed to the second half of his intended subject after briefly reviewing the other methods mentioned above. The references to recent literature in the following text are not necessarily comprehensive; they have been selected because they indicate certain important trends in analytical practice.

*Quantitative Spectrography.*—The large majority of workers in this field are broadly concerned with only two aspects of a spectrum, the position in it of a particular elemental line and the relative density of that line when recorded on a photographic plate. The accuracy with which the second of these measurements is being made is increasing steadily, and in recent work at the expense of the speed in making a determination. As regards the first measurement the spectrograph is established as a powerful tool in qualitative analysis. Two recent publications, one dealing with the analysis of high purity zinc and zinc alloys<sup>5</sup> and the other with metallurgical analysis,<sup>6</sup> describe clearly the amount of care and research necessary to obtain results of maximum accuracy. Much attention must always be paid to general technique, of which the photographic aspect is by no means the least important.<sup>7,8</sup>

Accurate assessment of the value of the quantitative spectrographic method involves three factors. As it is at present used, the spectrographer normally has some previous knowledge of the composition of the material he examines, and the method has been largely applied to analyses where this knowledge is largely implicit, *e.g.*, metallurgical analysis. Such previous knowledge may, of course, have been obtained by the use of the spectrograph but more frequently by other examination. Thus it has been observed<sup>9</sup>

<sup>1</sup> R. Belcher, "Microchemistry and its Applications", Monograph published by the Royal Institute of Chemistry, 1946.

<sup>2</sup> L. T. Hallett, *Ind. Eng. Chem. Anal.*, 1942, **14**, 956.

<sup>3</sup> H. Roth, *Angew. Chem.*, 1940, **53**, 441.    <sup>4</sup> G. H. Wyatt, *Chem. and Ind.*, 1942, **61**, 132.

<sup>5</sup> "Polarographic and Spectrographic Analysis of High Purity Zinc and Zinc Alloys for Die Casting", British Standards Institution Panel of the Non-Ferrous Industry Committee; H.M. Stationery Office, 1945.

<sup>6</sup> "Collected Papers on Metallurgical Analysis by the Spectrograph", edited by D. M. Smith; British Non-Ferrous Metals Research Association, 1945.

<sup>7</sup> E. H. Amstein, *J. Soc. Chem. Ind.*, 1943, **62**, 51.

<sup>8</sup> N. S. Brommelle and H. R. Clayton, *ibid.*, 1944, **63**, 83.

<sup>9</sup> W. Seith, *Deut. Tech.*, 1941, **9**, 254; *Chem. Zentr.*, 1941, **II**, 928.

that a combination of spectrographic and chemical methods is better and more reliable for the determination of impurities in zinc than other methods such as the polarographic.

Secondly, the spectrographer must be assured of a representative sample. Difficulties connected with this can to some extent be overcome by the facility with which many determinations can be made, but the Bureau of Mines in America,<sup>10</sup> for example, has found it necessary to develop methods for the preparation of samples to be used for both spectrographic and X-ray examination in the evaluation of dust hazards. Thirdly, the ability to make many determinations in a reasonable time allows a statistical survey of the results to be made. Such surveys, where an adequate number of results is available, are of recognised value.

The above points have been mentioned to draw attention to the tendency to use and regard the spectrograph as a testing rather than an analytical instrument. This is not surprising, for the natural advantages of the method—low sample consumption, automatic permanent record of results, speed in making many determinations, etc.—are all of great value where much routine testing has to be done. Nevertheless, the complexity of a spectrogram, the recognised interferences of elements present in the exciting source, and the by no means negligible influence on a spectrogram of the physical state of the material under examination all suggest that valuable information, additional to the amount of a particular element, might be obtained by further interpretation of a spectrogram. On these lines there is little progress to report.

*Colorimetric and Turbidity Measurements.*—It has been remarked that the absorptiometer is primarily of use in the quantitative analysis of certain solutions the composition of which is already qualitatively and possibly partly quantitatively known.<sup>11</sup> Such a statement applies equally well to most instruments used for the mechanical measurement of colour and turbidities and indicates the value of these instruments in repetitive work. For it is in this work that matching by means of a photoelectrical measuring device is more effectively done than matching by eye.<sup>12</sup> It is not surprising, therefore, that, having an instrument which will give the same reading for the same amount of a coloured substance, a great deal of attention has been paid to the development of measuring techniques<sup>13</sup> and to the preparation of selective organic reagents which produce highly coloured compounds.<sup>14</sup>

Methods involving turbidity measurements with some form of photo-electric cell have not made such strides. Certain satisfactory determinations have been recorded, such as the determination of zinc by measurement of the

<sup>10</sup> J. W. Ballard, H. I. Oshry, and H. H. Shrenk, *J. Opt. Soc. Amer.*, 1943, **33**, 667.

<sup>11</sup> H. K. Whalley, *Chem. and Ind.*, 1942, **61**, 495.

<sup>12</sup> A. Ringbom, *Chim. et Ind.*, 1941, **45**, No. 3 bis 304.

<sup>13</sup> Abstract review of lectures delivered at symposium of the Analytical Group of Verein deutscher Chemiker, *Die Chemie*, 1942, **55**, 361.

<sup>14</sup> J. G. N. Gaskin, *Ann. Reports*, 1945, **42**, 255.

fluorescent turbidity of the oxine complex<sup>15</sup> and the determination of small amounts of bismuth by measurement of the turbidity produced by the addition of bromate-bromide mixture.<sup>16</sup> Generally, however, accurate measurements of turbidities have increasingly revealed the extent to which small variations in conditions affect the turbidity, *e.g.*, barium sulphate<sup>17</sup> and barium carbonate.<sup>18</sup> In fact, barium sulphate figures are often inaccurate.<sup>19</sup> It becomes clear, then, that the introduction of the photo-cell into turbidity measurements could provide much valuable knowledge of the formation of precipitates, particularly up to the stage of coagulation.

*Polarography.*—The polarograph, as an instrument suitable for the accurate determination of small amounts of particular elements and compounds, is becoming more widely appreciated. Reviews of its uses, ranging from its elementary applications<sup>20</sup> to recent developments,<sup>21</sup> have been published. A panel of the British Standards Institution has produced recommended methods for the polarographic and spectrographic analysis of high-purity zinc and zinc alloys for die-casting, together with an account of the experimental work leading to the recommendations made. More recently, accounts have been given of applications of the polarograph,<sup>22</sup> its use in biochemical analysis,<sup>23</sup> in the analysis of aluminium, magnesium, and zinc<sup>24</sup> and of high-purity selenium, and compounds of nickel and cobalt.<sup>25</sup>

The authors of these four papers emphasise that the polarograph must be regarded as complementary to, rather than replacing, existing analytical methods. They find it difficult to generalise about polarographic problems; each problem has to be treated on its merits. Some polarographic methods are considered to be outstanding, *e.g.*, the determination of cadmium as an impurity in zinc. The polarograph is most easily adapted to routine testing, and it may be said that its potentialities in other directions have not been sufficiently examined because of this.

*X-Ray Diffraction.*—Hitherto in this account the different analytical methods described can and often do provide the same information, that is, the amount of an element in a given material. It is frequently a matter of personal choice whether micro- or macro-methods, spectrograph, or polarograph are used. The X-ray diffraction camera on the other hand provides information which the other methods (except sometimes indirectly) cannot, and herein lies its importance. Thus the determinations of 0.1% of calcium oxide in magnesium oxide, and of 0.2% of zinc oxide in zinc sulphide are quite feasible<sup>26</sup> and have been made. Similarly, X-ray diffraction studies of

<sup>15</sup> L. L. Merritt, jun., *Ind. Eng. Chem. Anal.*, 1944, **16**, 758.

<sup>16</sup> A. K. Majumdar, *J. Indian Chem. Soc.*, 1944, **21**, 157.

<sup>17</sup> W. Volmer and F. Fröhlich, *Z. anal. Chem.*, 1944, **126**, 401.

<sup>18</sup> J. G. N. Gaskin, unpublished.

<sup>19</sup> F. Canals and A. Charra, *Bull. Soc. chim.*, 1945, **126**, 89.

<sup>20</sup> J. G. N. Gaskin and H. K. Whalley, *Chem. and Ind.*, 1943, **62**, 441.

<sup>21</sup> J. E. Page, *Nature*, 1944, **154**, 199.

<sup>22</sup> W. Cule-Davies, *Analyst*, 1946, **71**, 49.

<sup>23</sup> J. E. Page, *ibid.*, p. 52.

<sup>24</sup> A. S. Nickelson, *ibid.*, p. 58.

<sup>25</sup> R. H. Jones, *ibid.*, 1945, **70**, 60.

<sup>26</sup> H. P. Rooksby, *ibid.*, p. 166.

paving asphalts<sup>27</sup> reveal information unobtainable by other means, and such materials meeting the same specification have been found to differ greatly. A complete review of the application of monochromatic X-rays to the analysis of mixtures has been published by M. Patry;<sup>28</sup> S. T. Gross and D. E. Martin<sup>29</sup> have also described the use of powder-diffraction methods for the analysis of crystalline mixtures. Attention has been drawn to the value of powder-diffraction analysis supplemented by and frequently preceded by spectrographic examination.<sup>30</sup> The preparation of suitable samples for X-ray work has already been mentioned.

*Macro-methods.*—For the purposes of this account two examples of the use of macro-methods in the determination of small amounts are of value. These are the published standard methods<sup>31</sup> for the chemical analysis of high-purity zinc and zinc alloys for die-casting whereby known standard metals can be provided for spectrographic and other purposes, and the successful adaptation of the method of H. H. Willard and O. B. Winter<sup>32</sup> to the determination of small amounts of fluorine in foods,<sup>33</sup> in coal and factory dusts, and in airs.<sup>34</sup>

*Modern Outlook.*—The great majority of the papers abstracted in the analytical sections of the various publications are concerned with either qualitative or quantitative examination for elements. Apart from organic analysis only one of the recently developed methods attempts to make determinations of compounds as such in a submitted material. Few papers relate the determined analytical figures with the physical state of the material examined.

Analysis for elements has been brought to a considerable state of perfection, so much so that the successful repetition of quite difficult determinations is a commonplace. This achievement, and it is an achievement, has undoubtedly been made possible by the introduction of the new physical methods, the spectrograph, the absorptiometer, and the polarograph. Nevertheless, it must be recognised that this ability to make numerous elemental determinations, as and when desired, and by the particular method favoured by the operator, does not constitute the full meaning of analysis or its complete purpose. Having made certain of his ability to determine the primary constituents of his material, the analyst must surely now desire to study its structure.

It has been recognised that a combination of chemical and physical methods can yield more information than either individually.<sup>35</sup> The com-

<sup>27</sup> C. L. Williford, *Agric. and Mech. Coll. Texas, Eng. Exp. Stat. Bull.*, 73, 70 pp.; *Road Abs.*, 1945, **12**, No. 4, 8.

<sup>28</sup> *Chim. et Ind.*, 1941, **45**, No. 3, 259; *Chem. Zentr.*, 1941, II, 3221.

<sup>29</sup> *Ind. Eng. Chem. Anal.*, 1944, **16**, 95.

<sup>30</sup> L. K. Frevel, *ibid.*, p. 209.

<sup>31</sup> British Standard Specification 1005, 1942; British Standards Institution.

<sup>32</sup> *Ind. Eng. Chem. Anal.*, 1933, **5**, 7.

<sup>33</sup> "Determination of Fluorine in Foods", Report of a Sub-Committee of the Analytical Methods Committees of the Society of Public Analysts, *Analyst*, 1944, **69**, 243.

<sup>34</sup> J. G. N. Gaskin, unpublished.

<sup>35</sup> W. C. Crone, junr., *The Frontier*, 1945, **8**, No. 4, 3/5 and 10/11,

plete realisation by the analyst that he must combine the information he gets from all of the main methods of examination (micro-, spectrograph, etc.) is a step in the right direction. Then, to render substantial assistance is the development of the X-ray diffraction camera which already yields information beyond the powers of existing analytical methods. Finally, certain possible developments of the other physical methods may help. Harnessed to the rigid necessity of performing its present qualitative and quantitative work, the spectrograph has to avoid different physical states in the exciting source, whereas such variation might be related to varying physical state. Similarly with line interferences. How the absorptiometer might assist has already been indicated. The polarographer usually wishes to suppress unwanted maxima in his diffusion currents and does so with "surface active" substances. Attempts have already been made to use the suppression of these maxima to measure the quantity or indicate the presence of such compounds. Further, the polarograph can be used to prove the presence of and determine the amounts of compounds where these are reducible at the dropping electrode.

It must be recognised that this analysis for compounds and the determination of physical state is of fundamental importance. A single example will show this. Despite the tremendous amount of work which has been done in the evaluation of dust hazards, the determination in a dust of the kind and amount of silica which causes silicosis is a problem which as yet is not completely solved. The X-ray diffraction camera is providing new information, but for that to succeed preliminary and complementary work using many methods will be necessary. Here then is the present and future position of the analyst. He can provide all elemental information. He will provide information as to compound constituents and their physical state.

It would be wrong in this review not to draw attention to a matter which has been frequently discussed in recent times, *viz.*, the necessity of improving analytical instruction in this country. It is hoped that the Reporter has made it clear what is to be expected of an analyst. Such an analyst would require a very wide scientific analytical knowledge. Where is he to get it?

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